AN ABSTRACT OF THE THESIS OF

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Title: Translocation of Photosynthates in Soybeans (Glycine Max. (L) Merr. cv. Wilkin) as Affected by Soil Water Potential and Soil Temperature

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Experiments determined effects of soil water potential of -0.35, -2.5, and -5.0 bars and soil temperature of 25 and 10°C on osmotic potential and turgor potential of leaves and on translocation of ^{14}C-labeled photosynthates in 18-day old soybean seedlings (Glycine Max. (L) Merr. cv. Wilkin). Decrease in soil water potential from -0.35 to -5.0 bars decreased leaf water potential from -7.3 to -13.5 bars, osmotic potential corrected for apoplastic water content from -13.8 to -17.8 bars and turgor potential from 6.5 to 4.3 bars. Lowering soil temperature from 25 to 10°C decreased leaf water potentail, osmotic potential, and turgor potential by 2.4, 1.6, and 1.0 bars respectively.

Elongation rates of second trifoliate leaves decreased from 11.0 to 1.9 mm/day, those of third and fourth trifoliate leaves from 13.3 and 1.3 mm/day to no growth when soil water potential decreased from -0.35 to -5.0 bars at 25°C. All expanding leaves ceased to elongate at -2.5 and -5.0 bars at 10°C.

Seedlings equilibrated with ambient environment in continuous light at 400 $\mu E/m^2/sec$ in photosynthetic range for two days before start of translocation experiments. Fully expanded first trifoliate leaves were pulse labeled for 5 minutes with air containing 8.0 ppm

by volume of $^{14}CO_2$ drawn at the rate of 1.5 l/min. Activity in labeled leaves, second trifoliate leaves, tips, stem, roots, and nodules was determined at 1, 2, 4, 8, 12 and 24 hours. $^{14}CO_2$ fixation was reduced by 20 and 33 percent when potential decreased from -0.35 to -2.5, and -5.0 bars respectively. Plants at 10°C fixed 30 percent less $^{14}CO_2$ than at 25°C. Rates of export from labeled leaves were high during first two hours after labeling, then decreased. Activity in labeled leaves at 24 hours was 42, 52, and 58 percent of total dpm/mg at -0.35, -2.5, and -5.0 bars respectively and 60, 64, and 71 percent at 10°C.

Tips of plants at -0.35 bar and 25°C accumulated ^{14}C at faster rates than at -2.5 bars during the first 8 hours after labeling. Rates of ^{14}C accumulation were lower during later hours. Similar patterns were observed for other plant parts. At 24 hours, 14.8, 8.1 and 6.0 percent of total dpm/mg were in the tips at -0.35, -2.5, and -5.0 bars respectively and 4.9, 4.9, and 2.1 percent of total dpm/mg at 10°C. Stems at -0.35 bar accumulated more ^{14}C than those at -5.0 bars at all times after labeling. At 24 hours activity was 15.1, 12.3, and 12.5 percent of total dpm/mg at -0.35, -2.5, and -5.0 bars respectively.

At 25°C a decrease in soil water potential from -0.35 to -5.0 bars decreased ^{14}C imported by the roots at all times except at 24 hours when activity was 12.7, 13.3, and 11.5 percent of total dpm/mg at -0.35, -2.5, and -5.0 bars respectively and 6.5, 6.3, and 5.2 percent of total dpm/mg at 10°C.

At 24 hours activity in the nodules was 13.3, 12.5, and 10 percent of total dpm/mg at -0.35, -2.5 and -5.0 bars respectively. Absolute activity in the nodules expressed as dpm/mg was consistently lower at 10°C than at 25°C.

Translocation of Photosynthates in Soybeans (*Glycine Max*. (L) Merr. cv. Wilkin) as Affected by Soil Water Potential and Soil Temperature

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INTRODUCTION

The growth and productivity of plants results from the complex interactions between physical, chemical and biological processes, mediated by the environmental factors above and below the soil surface. Soil water content and soil temperature are the most important soil physical properties that affect plant growth and development. When soil water availability becomes limiting, plant water stress is increasingly pronounced, and low plant productivity and crop yield are the inevitable consequence.

In recent years tremendous efforts have been put into the study of the response of plants to water stress. The effects of water stress on net photosynthesis and growth of various plant tissues received much attention, but translocation of photosynthates, the linkage between the two processes, seems to have attracted fewer investigators. This is surprising since the well accepted pressure flow hypothesis, for the mechanism that drives the phloem translocation, refers to the turgor pressure gradient in the phloem resulting from influx of water from the xylem as a driving force. The quantitative assessment of translocation of assimilates at known soil and plant water potentials is necessary in order to relate translocation to other physiological and biological responses at similar water stress levels. With this kind of information, linkages among different processes in the plant can be established. The mechanisms which plants utilize for physiological adaptation to water stress may

be better understood. Hopefully, the breeding and selection for adaptation of plants to water stress would be facilitated by a better knowledge of translocation.

Soil temperature is expected to affect translocation of photosynthate either by lowering the leaf water potential or by retarding the unloading of photosynthates in the roots. A decrease in rate of growth due to low soil temperatures is well known. The reduction in water and ion uptake, root growth, and net photosynthesis due to low soil temperatures has been demonstrated, but very little attention has been given to the study of changes in the distribution patterns of photosynthates at sub-optimum soil temperatures.

The objectives of the experiments reported here were to (i) examine leaf water potential, osmotic potential, turgor potential and growth characteristics of soybean seedlings as a function of soil water potential and soil temperature and (ii) examine the effects of soil water potential and soil temperature on translocation and distribution of photosynthates in soybean seedlings.

LITERATURE REVIEW

Photosynthates are synthesized in chloroplasts and translocated throughout a plant in phloem tissues. Carbohydrate is the main substance being translocated from the sites of production in the mature leaves to the sites of utilization and storage in the growing tips, roots, and storage organs.

References on translocation of photosynthates are voluminous and these subjects have been reviewed in detail (Crafts and Crisp, 1971; Canny, 1973; Zimmermann and Milburn, 1975; Aronoff et al., 1975; Wardlaw, 1974; and Baker, 1978). The purpose of the present literature review is to highlight the significance of the phloem solution, the mechanism by which it moves, and the quantitative methods for assessing translocation. More detailed reviews about the environmental factors affecting transport of assimilates, particularly water stress and temperature, are presented.

Composition of Phloem Sap

Analytical studies of phloem exudates from many plant species indicate that sucrose is the main form of carbohydrate in the phloem. It may account for over 98 percent of all sugars present in sieve tube exudates of plants such as Salix viminalis (Peel and Weatherly, 1959). In some species other sugars such as raffinose, stachyose, and occasionally the sugar alcohols mannitol and sorbitol may occur. There are several reasons for the predominance of the above substances in the phloem. Sucrose and sugar alcohols are easily synthesized from the early products of photosynthesis without large expenditures of energy. They are highly soluble, protected against

enzyme breakdown during transport (Arnold, 1968), and suitable for active transport through membranes.

Nitrogenous substances are much less concentrated in phloem exudates than carbohydrates in most species. The most widespread nitrogenous compounds are the glutamic and aspartic acids, glutamine and asparagine. However, the concentration of those amino acids and amides are not constant throughout the year (Mitler, 1958) and increase during leaf senescence (Ziegler, 1964).

A number of inorganic ions are found in phloem saps. The predominant cation is K^+. It is readily redistributed in the plant and has an important role in the postulated electro-osmotic flow mechanism that may drive phloem transport (Spanner, 1970). Recently, Smith and Milburn (1980) interpreted an increase in K^+ levels in the phloem as an osmoregulatory response to conditions of restricted sucrose availability such as during darkness. Magnesium is also translocated in phloem but in lesser amounts than K^+. Calcium occurs only in minute concentrations in the sieve tube saps. The presence of high concentrations of K^+ and Mg^{++} in the phloem explains the first appearance of deficiency symptoms of these two elements in the older leaves. When K^+ and Mg^{++} supply is limited these elements will be transported from the older to the younger plant parts. To the contrary, calcium deficiency first appears in the younger leaves due to its immobility in the phloem.

The most common inorganic anions are Cl^- and PO_4^{3-} with trace amounts of SO_4^{2-} and HCO_3^-. But NO_3^- is absent from the phloem sap (Baker, 1978). The total amount of inorganic anions is less than that of cations. Charges are, however, balanced by organic anions.

Organic acids are quantitatively a minor constituent of phloem exudates. Malate has been found to be transported in the sieve tubes of soybeans (Nelson et al., 1961).

Analyses of phloem exudates by Kluge and Ziegler (1964) in many tree species reveal a relatively high concentration of ATP. Similar observations have been reported by Gardner and Peel (1969) and Hall and Baker (1972) for other species. Naturally occurring growth substances have been detected in phloem sap. It is apparent that these substances, which are water soluble and cannot be synthesized in the non-green tissues of the plants, are translocated along the sieve tubes in varied concentrations. Additional details on the nature of transported substances have been presented by Ziegler (1975) in a very complete review.

Direction of Movement of Assimilates

Assimilates move from the photosynthesizing leaves (sources) to the stem and then upwards as well as downwards towards "sinks" such as the roots and storage organs. Experiments on distribution patterns of photosynthates for various plant species (Thrower, 1962; Wardlaw, 1968) lead to the generalization that lower leaves of a plant export primarily to the roots, upper leaves export to the shoot apex, and the leaves in between export in both directions.

Detailed studies on translocation patterns in soybeans (Glycine max. (L.) Merr.) by Thaine et al. (1959) show similar results. The direction of movement of assimilates from a given soybean leaf depends upon the age and position of the leaf on the stem. The young leaf behaves as a major apical sink for upward movement of assimilates

without any export to other parts of the plant. The expanding leaf changes its role from importing to exporting when it reaches about 50 percent of its maximum size. The shoot apex and roots are the main sinks for materials being transported. Movement into primary and mature trifoliate leaves was very small compared to movement into tissues of expanding leaves.

Loading and Unloading of the Phloem

There are two parallel pathways through which loading of sugar from the mesophyll into the phloem can take place, one being the symplastic pathway and the other the apoplastic pathway. Sucrose moves from the chloroplasts in the mesophyll cells mainly by diffusion via the symplast (Tyree, 1970) to the phloem of the minor veins where it enters the free space. Alternatively, sugar may move from the mesophyll cells to the phloem by diffusing through either the apoplast or the extracellular spaces (Geiger et al., 1974). Details of these pathways have been described in Geiger (1975). Sucrose is actively loaded against an electrochemical potential gradient from the free space through the plasmalemma of sieve tube companion cells or transfer cells at the source region. Increases in solute concentrations inside companion cells or transfer cells in some plant species induces influx of water from the free space due to differences in osmotic pressures. Resulting pressure gradients move assimilates into the sieve elements and down the sieve tubes. In the sink regions, the process is reversed with efflux of sucrose and water from the companion cells or transfer cells into the symplast of the sink.

Anderson (1974) suggested that the symplastic pathway functions during daylight hours because high sugar concentrations in the mesophyll increase the concentration difference between chloroplasts and sieve elements, hence accelerate the diffusion process. The apoplastic pathway is powered by ATP derived from respiration and functions equally well during hours without light.

Phloem loading depends on metabolic energy (Sovonik et al., 1974). Loading of sugars from the free space is promoted by exogenously applied ATP and is sensitive to metabolic inhibitors. Recent studies by Giaquinta (1977), Malek and Baker (1977), and Humphrey (1978) demonstrated that the sugar loading is a proton co-transport system. In this system, a proton gradient across the plasmalemma is created and maintained by ATPase, which is assumed to act as a proton pump in the plasmalemma. The energy from ATP is utilized for pumping protons out of the cell against an electrochemical potential gradient. The sucrose and H^+ co-transport occurs along this gradient resulting in active influx of sucrose into the sieve elements.

Translocation Mechanisms

Although sugar transport in phloem occurs along a concentration gradient, the rate of movement is such that the apparent diffusion coefficient is ten thousand times larger than the measured values in aqueous solution (Crafts and Crisp, 1971). This denies the diffusion process as the driving force and favors a mass flow mechanism for the translocation stream. The questions of what the moving stream consists of and how it is driven are highly controversial. Many models have been proposed to explain the translocation process. Only a few

of these will be briefly discussed. More details can be found in reviews by Zimmermann and Milburn (1974) and by Aronoff et al. (1975).

Pressure Flow Hypothesis

The simplest, but among plant physiologists most widely accepted, mechanism for driving phloem flow is the pressure flow hypothesis originally suggested by Münch (1930). This hypothesis postulates that loading photosynthates, produced in the source leaves, decreases osmotic potential in the sieve tubes thereby inducing the entry of water in the phloem which in turn creates a higher turgor pressure. Unloading of sugars from the phloem in the sink areas, with a corresponding efflux of water from the sieve elements decreases turgor pressure. Thus a turgor pressure gradient is established which drives the flow of photosynthates from the source to the sink regions. The existence of a turgor pressure gradient in the sieve tubes has been reported for several varieties of plants (Roger and Peel, 1975; Sheikholeslam and Currier, 1977a; Lee, 1981; and Fisher, 1978). The evidences for and against the Münch hypothesis were summarized by Weatherly (1975).

Electro-osmotic Model

The electro-osmotic model for phloem flow was proposed independently by Fensom (1957) and Spanner (1958). According to this model, sugar solution is driven through the sieve pores and along the sieve tubes by electro-osmotic drag of K^+ ions which circulate from the companion cells through the sieve plate back into the companion cells. MacRobbie (1971) critically reviewed this model and her calculations showed that requirements for metabolic energy and K^+ fluxes are too high to accommodate this flow mechanism.

Peristaltic Tubule Model

This model was postulated by Thaine (1964) and modified by Aikman and Anderson (1971). Rhythmic contractions of microtubules, running from sieve element to sieve element through the sieve pores, generate a flow of assimilates along these tubules. Despite its hydrodynamically and thermodynamically efficient mechanisms, the model falls short of anatomical evidence to support the presence of microfibrils in the sieve tubes.

Protoplasmic Streaming

The features of this theory are that a network of P-protein fibrils is thought to be responsible for generating a motive force for driving bulk flow of the sieve flow contents. MacRobbie (1971) discussed the possible ways in which fibrillar P-protein might be organized in the sieve elements. Although this model is anatomically attractive, its main disadvantage is that simultaneous movement of solutes in opposite directions within a single sieve tube cannot be explained by this mechanism. The continuous energy input along the sieve tube is required in this theory. If this model is correct, the insensitivity to cooling of the translocation pathway in certain plant species cannot be explained.

Summary

There appears to be no single model that can completely satisfy physical laws of flow, physiological processes observed in the system, and structure of the sieve elements. However, there seems to be stronger evidence to support the Münch pressure flow hypothesis than the others. The actual mechanisms driving the phloem translocation

stream will probably be controversial until the fine structure of the sieve elements is completely understood.

Estimation of Rate of Translocation

Several techniques are available for evaluating the translocation process quantitatively. The choice of a particular method depends on plant species under study and equipment available. A summary of methods that are commonly employed in translocation studies follows.

Incision Method

The stem of a plant sample is either cut or punctured and exudate from the cut area is collected and chemically analyzed for contents and concentration. This is the simplest and the most common technique used in the early work on phloem translocation. When concentrations from different heights on the trunk of the trees are determined, velocities of the flow can be estimated by tracking the moving wave of a concentration ratio between different species of sugars in phloem (Zimmermann, 1969). A limitation of the incision method is that not all plant species provide exudates. Repeat cuttings of the tissue are required to maintain the flow of sap in some species. Moreover, incision is not precise and the phloem sap can be contaminated with the contents of cut parenchyma cells.

Aphid Stylets

The pioneers in utilizing severed stylets of various species of aphids were Kennedy and Mitley (1953). In this technique, a colony of aphids is allowed to feed on the stems of the host plants. Aphids are

then anaesthesized and their stylets severed. Exudate is collected from the cut ends of the stylets left embedded in the host plant. This method provides useful information about the nature of substances within the phloem. Although it has been employed by many investigators (Weatherly et al., 1959; Zimmermann, 1961; Evert et al., 1968) more recent reviews of this method (Dixon, 1975; Peel, 1975) agree that damage done on host plants by aphids limits the application of this technique to phloem translocation studies.

Radiotracer Method

There are two methods involving the use of radioisotopes and counting instrumentation for translocation studies.

Steady State Labeling. This method requires the exposure of plant samples to the tracer material throughout the entire period of the experiment. The total amount of carbon in the material of interest can be estimated from the known ratio of one atom of ^{14}C to total amount of carbon atoms in the compound being supplied to the plants. This method is not commonly used since it requires specially designed systems to maintain steady state labeling and does not permit certain measurements on the plant samples.

Pulse Labeling. The most widely employed tracer method in phloem translocation studies has been pulse labeling. In this method, a labeled substance is introduced into the transport system, usually via a mature leaf, for a relatively short fixation period and is then withdrawn. The activity of labeled carbon in other parts of the plant is determined after known intervals of time. Translocation rate and velocity may be estimated. A labeled ^{14}C is commonly used as the tracer

due to its commercial availability. The isotope ^{11}C with the shorter half-life was used by Moorby et al. (1963). The use of ^{11}C is advantageous because a number of experiments can be performed on the same plant by allowing the ^{11}C to decay between treatments. On the other hand, the need for a special activator to produce ^{11}C prevents most laboratories from using this isotope.

Carbon Balance Method

Estimates of translocation rates of carbon from a leaf can be achieved by the carbon balance equation,

$$\text{Rate of Translocation} = \text{Rate of Net Photosynthesis} - \text{Rate of Night Respiration} - \text{Rate of Growth} \quad (1)$$

The terms on the right hand side of the equation can be determined and the rate of translocation can be calculated. Hopkinson (1964) measured dry weight and CO_2 exchange of similar cucumber leaves and determined the rate of translocation by the difference. Realizing the important comparability between leaves, Terry and Mortimer (1972) determined CO_2 exchange and growth rates on the same leaf. Estimation of carbon accumulation and loss from changes in dry weight was done by using leaf discs with a known area and assuming that over a short duration the proportion of carbon in the dry matter was 42.1 percent. Silvius et al. (1978) reported agreement between translocation rates obtained from this method and results from labeling experiments.

The carbon balance method seems to be attractive because it offers a direct way to estimate rate of translocation. However, dry weight changes during short periods are difficult to determine precisely. This method is, therefore, appropriate for longer translocation periods

such as 24 hours, as used by Turgeon and Webb (1975) or 10 hour periods as used in the work of Silvius et al. (1978).

Effects of Water Stress on Translocation

According to the pressure flow hypothesis of phloem flow, water is osmotically induced into the sieve tubes following loading of photosynthates at a source region. A steady state mathematical model of pressure flow (Tyree et al., 1974) shows the effects of water potential, solute concentration, and pressure gradient along the sieve tubes on phloem translocation. If the pressure flow hypothesis is correct, translocation of assimilates is expected to be reduced by lowering plant water potential.

The literature on translocation processes as affected by water stress reveals variations in degree of responses among plant species. Hartt (1967) reported a more severe reduction in rate of translocation of photosynthate than in rate of photosynthesis in water stressed sugar cane. The same phenomenon was found in corn plants by Brevedan and Hodges (1973). However, Munns and Pearson (1974) found that the translocation rate expressed as a percentage of total ^{14}C activities (% total dpm/hr) over a 24 hour period was not reduced when potato plants (Solanum tuberosum) were stressed. However, the absolute rate of translocation (dpm/hr) from the leaves decreased with decreasing water potential due to the marked decline in net photosynthesis in the water stressed plants.

Responses in other plant species are somewhat different. Hoddinott et al. (1979), using a steady state ^{14}C labeling system to estimate translocation rate in Phaseolus vulgaris subjected to different concentrations of osmoticum, reported that the translocation rate is less

sensitive to osmotic shock than the photosynthetic rate. This trend was also observed in cotton (Gossypium hirsutum L.) and sorghum (Sorghum bicolor L.) by Sung and Kreig (1979), and in semi-dwarf wheat (Triticum aestivum L.) by Johnson and Moss (1976).

Attempts have been made to clarify the stages of translocation which are affected by water stress. Plaut and Reinhold (1965) concluded from a labeling experiment on Phaseolus vulgaris that water stress retards assimilate movement within the phloem itself in addition to its effects on phloem loading. This conclusion was based on results which indicated reduction in ^{14}C activities expressed both as absolute activities and as percentage of total ^{14}C activities in all plant parts from mid-vein to roots. In contrast, results from the experiment with Lolium temulentum L. performed by Wardlaw (1969) suggested that the response to water stress did not result from direct effects on the transport process, but rather from effects on growth, photosynthesis, and loading of assimilates into the sieve tubes. In a more recent paper Watson and Wardlaw (1981) described the use of steam to kill the tissues at the base of the blade of several C_3 and C_4 plant species in order to mimic the possible effect of reduced demand for leaf photosynthate resulting from reduced growth due to water stress. Steam treatment reduced partitioning of ^{14}C in wheat and sorghum. The proportion of ^{14}C entering the amino acid fractions was increased in wheat but no change in amino acid fraction was detected in sorghum. However, defoliation of the adjacent mature leaves in order to simulate an increase in demand for photosynthate of the labeled leaves did not enhance the export of ^{14}C-photosynthate from the leaves of either well watered or stressed wheat and sorghum. They concluded that the

reduction of ^{14}C translocation due to water stress was independent of the amount of competing photosynthetic tissue available for growth. The question of whether translocation is directly affected by water stress or by the reduction in growth due to stress conditions is not completely resolved. To do so requires a more adaptable and accurate way of determining turgor pressure of a single sieve tube, assuming that pressure flow is the driving mechanism of the assimilates in the phloem tissue.

At present, the most direct method of measuring turgor pressure of sieve tubes is the needle technique (Hammel, 1968). Using this method, Sheikholeslam and Currier (1977b) showed that a reduced translocation rate in a water stressed squirting cucumber (Ecballium elaterium was associated with a lowering of the turgor pressure gradient along the translocation pathway. Sovonick-Dunford et al. (1981) modified the Hammel-type phloem needle by connecting it to a pressure transducer and used it to measure phloem turgor pressure in white ash (Fraxinus americana L.). Clear diurnal changes in phloem turgor were observed. Values reached a minimum at midday and a maximum in the early morning corresponding to low and high xylem water potentials respectively.

There are some problems inherent in the phloem needle technique, namely the relatively large size of the needle compared to the size of sieve elements and the destruction of a large number of cells when the needle is inserted (Sovonick-Dunford et al., 1981). Moreover, the size of the needle limits the use of this technique to trees since the stems of most plants are too small for reliable measurements to be made.

Indirect measurements of phloem turgor pressure may be obtained from the algebraic difference between xylem water potential and phloem osmotic potential assuming an equilibrium existed between the xylem and phloem water potential at the time of measurement. The difficulties are with the methods for determining osmotic potential. Attempts have been made to measure this value either by collecting sieve tube sap from severed aphid stylets or from incisions made in the phloem (Roger and Peel, 1975), and by negative staining procedures (Fisher, 1978). However, errors or assumptions inherent in measuring both water and osmotic water potential in the phloem have not been resolved. More complications are expected if one wants to determine the phloem turgor pressure gradient between a source leaf and an active sink such as growing roots because of the greater difficulty in measuring xylem water potential in roots.

One aspect of studies of the effects of water stress on assimilate translocation that deserves more consideration is the control of treatment levels applied in the experiment. Two groups of plants have been frequently used as experimental materials, with one being well watered and the other grown on soils which are allowed to dry enough to produce stressed conditions (Munns and Pearson, 1974; Plaut and Reinhold, 1965; Silvius et al., 1977; Brevedan and Hodges, 1973; Johnson and Moss, 1976). Translocation rates are often estimated from radioactivities of ^{14}C at certain periods up to 24 hours after labeling during which leaf water potential is subjected to changes due to decreasing soil water potential and evaporative demand.

More controlled treatments have been imposed by growing plants in solutions with different osmotic potentials (Hartt, 1967;

Sheikholeslam and Currier, 1977a). The lack of good aeration of the submerged roots might confound treatment effects on rate of translocation as suggested by the work of Nuritdinov and Vartapetyan (1976) who reported that even the short deprivation of oxygen in the root zone of cotton could significantly affect the rate of transportion of sugars from leaves to roots.

Summary

In spite of a large number of papers on translocation of photosynthates under water stress, there appears to be uncertainty about the degree of response among different plant species. Even within a certain species, it is difficult to relate results from one experiment to others due to the qualitative nature of the treatments applied. Plant water potentials were often not reported. The evidences for direct influence of xylem water potentials upon phloem water potentials suggest a more careful setup of experimental conditions to avoid significant changes in plant water potential during translocation measurements in order to obtain more meaningful results.

Other Physiological Effects Due to Water Stress

Apart from aforementioned effects of water stress on translocation of photosynthates, other physiological effects have been exhaustively investigated. The excellent reviews on this subjects can be found in Hsiao (1973), Slatyer (1967) and Turner and Begg (1981). The emphasis of the following review will be on growth, photosynthesis, and other processes which associate either directly or indirectly with translocation processes.

Growth

Evidences from experiments with many plant species have suggested that leaf expansion has been one of the processes most sensitive to water stress. Boyer (1970a) observed a complete suppression of the enlargement of sunflower at leaf water potentials below -4 bars. The rates of leaf enlargement in soybeans and corn were 25 and 20 percent of the observed maximum rate of enlargement respectively, at leaf water potential of -4 bars in his growth chamber experiments. Barlow et al. (1976) found a 44% reduction in the leaf elongation rate of corn seedlings when soil water potential was decreased from -0.35 to -2.50 bars. In the study designed to compare leaf elongation rate, leaf water potential, and turgor pressure of soybeans growing in a growth chamber, glass house, and in pots outdoors, Bunce (1977) showed that there was a linear relationship between elongation rate and turgor pressure in all three environments. The threshold of turgor pressure was between 0.2 bar to 1.5 bars in outdoor and growth chamber-grown plants, respectively. In contrast, mean elongation rates of field grown soybean plants were found not to be affected by midday turgor potentials during the vegetative growth stage (Wenkert et al., 1978a). There seems to be general agreement that leaf expansion is much more sensitive to water stress than photosynthesis (Boyer, 1970; Barlow et al., 1977; Turner and Begg, 1981). Leaf elongation rate is often higher at night or under shaded conditions than during a clear day (Bunce, 1977; Wenkert et al., 1978a). The rate of expansion can recover to become close to that of well watered plants if the level of water stress experienced

by plants is not too severe or prolonged (Boyer, 1970a; Acevedo et al., 1971).

Photosynthesis

Water stress can influence the rate of photosynthesis of plants either by reducing leaf area or by decreasing rate of net photosynthesis per unit leaf area or both. The evidence for reduction in rate of leaf elongation under water stress is well documented as previously discussed. The rate of net photosynthesis rate has frequently been reported to be less sensitive to low leaf water potential than the rate of leaf expansion (Barlow et al., 1977; Turner and Begg, 1981). The strong correlation between stomatal resistance and rate of net photosynthesis at various leaf water potentials (Boyer, 1970b; Hansen, 1971; Slatyer, 1973) lead them to conclude that stomatal resistance may account for much of the decrease in photosynthesis. However, data from Barlow et al. (1977) show an increase in resistance of both mesophyll and stomata with a decrease in leaf water potential. This suggests that internal physical and biological as well as stomatal conditions play important roles in mediating effects of water deficits on rates of net photosynthesis. Accumulation of starch was observed by Ackerson and Hebert (1981) to severely limit rate of photosynthesis in leaves of a cotton species which is known to adapt to water deficit.

Osmotic Adjustment

Accumulation of solutes in higher plants in response to water deficits is referred to as osmotic adjustment. The phenomenon has been shown in leaves, roots, hypocotal, and reproductive organs of

many plant species as fully reviewed by Turner and Jones (1980). This mechanism enables plants to withstand moderate water deficits and sustain growth during periods of limited water availability. The nature of the compounds involved in osmotic adjustment have been investigated in several plant species. Jones et al. (1980) reported that accumulation of sugars (glucose and sucrose) and inorganic ions (K^+ and Cl^-) contributed equally to the decrease in osmotic potential of fully expanded sorghum leaves grown at predawn leaf water potential of -0.85 MPa. Increases in the concentrations of K^+, Ca^{2+}, Mg^{2+}, NO_3^- and amino acids were observed in fully expanded sunflower leaves under water stress but sugars did not make any contribution to observed decreases in osmotic potential. The contribution of Cl^- to osmotic adjustment in winter wheat was observed by Christensen et al. (1981). Spring application of NH_4Cl fertilizer at the rate of about 350 kg Cl/ha reduced osmotic potential by as much as 4 bars in the leaves of two wheat varieties in their studies. Interestingly, calculated values of osmotic potential due to ions in the symplast using the van't Hoff equation correlated well with the decrease in osmotic potential found on the chloride treated plots.

The importance of low molecular weight solutes to osmotic adjustment in leaves of tropical pasture species was demonstrated by Ford and Wilson (1981). Sodium, potassium, and chloride ions were the most important solutes involved in decreasing the osmotic potential while sugars and organic acids contributed little to osmotic adjustment of the grass species in these studies. On the other hand, data from Ackerman (1981) showed that glucose and starch contribute to osmotic adjustment in water stress adapted cotton species.

The mechanism by which soluble sugars are accumulated in water stressed leaves is not clear. A decrease in translocation of carbohydrate from the leaves, an increase in starch hydrolysis, or a decrease in conversion of starch to other products may result in the observed sugar accumulation.

Effects of Temperature on Translocation of Assimilates

Rate of translocation is affected by temperature since loading and unloading processes and certain mechanisms proposed for driving assimilate flows along the sieve tubes require metabolic energy. Results from numerous experiments on temperature effects suggest the existence of two groups of plants according to their responses to low temperature treatments. The chilling-sensitive plants show a threshold where an abrupt decrease in translocation occurs when the temperature of stems or petioles is lowered below 12°C. In contrast, with chilling insensitive plants the threshold occurs at the temperature of 0°C or lower (Geiger and Sovonick, 1975).

A number of investigators subjected entire plants to different temperatures. Hewitt and Curtis (1948) summarized from their studies on beans and milkweed (Asclepia syriaca L.) that the rate of translocation from leaves was optimum between 20 and 30°C. Thrower (1965) spot fed the terminal leaflet of the upper expanded leaf of soybeans with $^{14}CO_2$ while the air temperature was held at 2-3°C. The radioautographs of the plants harvested 3 hours after labeling showed that $^{14}CO_2$ uptake in the labeled leaf was not severely inhibited by low temperature but that translocation from this leaf was restricted. Results from whole plant treatments, although providing useful information, do not

differentiate responses of various parts of the translocation system. The more preferable approach is to treat the plant locally with the design temperature. Temperature treatment of the source leaf separate from the rest of the plant presents difficulties in interpreting the results, because the rate at lower temperature may be due to decreased synthesis of photosynthates as well as to inhibited phloem loading.

Many reports about translocation of assimilates at different temperatures come from experiments involving localized path treatments. One of the most comprehensive studies in this area is that conducted by Webb and Gorham (1965). Using 13 day old, straight-necked squash plants (Cucurbita melopepo), they labeled the mature, primary leaf blades with $^{14}CO_2$. The primary node and basal end of the primary petiole were subjected to temperatures ranging from 0 to 55°C while the rest of the plant was kept at about 23 to 25°C. Results from the experiments indicated that the maximum rate of ^{14}C movement through the node occurred at 25°C. The translocation rate declined above and below this temperature with complete inhibition of ^{14}C transport at 0 and 55°C. When the temperature was rapidly raised from 0 to 25°C the rate of export from the blade began immediately and returned to the rate normally found at 25°C, 60 minutes after the temperature was raised. They did not detect significant changes in the $^{14}CO_2$ assimilation rate and transpiration rate of the leaf blade with these treatments. The subsequent study by Webb (1967) demonstrated that different sections of the translocation path i.e., stem petiole, hypocotyl, and nodes respond similarly to temperature.

In chilling-insensitive plants such as sugar beet, translocation continues undiminished after a short period of inhibition caused by

localized path chilling. The initial decline in export rate reverses back to the pretreatment rate after 1 to 2 hours of cooling (Swanson and Geiger, 1967). The chilling insensitive plants do not show drastic decrease in translocation rate.

The contrasting patterns of response by the two classes of plants led Giaguinta and Geiger (1973) to investigate the mechanism of inhibition of translocation by localized chilling. They compared Arrhenius plots of the temperature dependence of translocation velocity and mass transfer rate in sugar beet and beans. For beans, a chilling sensitive plant, a Q_{10} of 1.3 for temperatures between 10 and 25°C and Q_{10} of approximately 6.0 below 10°C were found whereas in sugar beets, a chilling insensitive plant, a Q_{10} of 1.3 was shown throughout the same temperature range. A Q_{10} of 1.2 to 1.5 indicates a mechanism limited by a physical process. Their cytological data also suggest that inhibition of translocation by chilling results from physical blockage of sieve plates, not from the direct inhibition of metabolic process which drive translocation.

Unlike the path treatment, chilling of sinks such as growing tissues, developing storage organs, or other metabolically active tissues attracted only a small number of investigators. The reason is probably that greater difficulty is encountered in setting up the controlled treatments than with localized chilling of the translocation path and/or the whole plant. Husain and Linck (1967) chilled the shoot apex of Pisum sativum L. and observed that translocation of foliar applied ^{32}P to the shoot tip decreased. But increased ^{32}P activity was detected in the roots. Geiger (1966) demonstrated that cooling the sink leaf of sugar beets to 1°C, not only reduced the

rate of translocation to approximately 40% of the pretreatment rate but altered the transport pattern of the assimilates also. The rate of import of photosynthates to a young uncooled leaf was increased by 30 percent. The data suggest that the translocation process involves active uptake into storage and growing tissue. Reports about the effect of root temperature on rate of translocation of assimilates are rare. Hartt (1965) studied photosynthate transport of sugar cane grown in nutrient solutions at different air and root temperatures and showed that the root temperature of 17°C decreased rate of translocation from the fed leaf at high light intensity. She also concluded that it could have been caused by increased moisture stress due to low root temperature. Unfortunately, no quantitative value of plant water stress was reported. Somewhat different responses to root temperature in wheat were observed by Rovira and Brown (1973). They found that the total amount of assimilate transported to the roots was not affected by root temperature. However, lowered root temperature, particularly to 5°C, resulted in slower movement of assimilate along the root thus allowing greater movement of assimilate out of the phloem into root cortex cells.

Literature reviewed so far suggests that effects of sink temperature on translocation rate and on distribution patterns deserve further investigation in order to interpret the effects of temperature on the whole plant correctly and to provide better understanding of effects of low root temperature on shoot growth and mineral accumulation in plants.

Root Temperature Effects on Other Physiological Processes

One of the main functions of plant roots is to extract water and nutrients from the soil. Roots are also the sites for nitrogen assimilation processes and for production of plant growth metabolites. The mechanisms by which temperature affects the uptake of water and nutrients was carefully analyzed by Ungs et al. (1982) using principles of thermodynamics and enzyme kinetics. Their models predict an initial increase in active solute uptake by the root system with an increase in temperature up to the optimum temperature where the rate of active solute uptake is maximum. At higher temperatures the active uptake flux decreases due to conformational changes of associated enzymes.

Dalton and Gardner (1978) derived a general equation for the effect of temperature on water and solute uptake by plant roots. Their equation suggests an increase in flux of water and solute uptake with temperature and the predicted values agree well with the experimental results from Kramer (1940). Other investigators also found a reduction in water and ion uptake when root temperature is decreased from the optimum level (Nordin, 1977; Markhart et al., 1979; Young, 1977).

Growth of both subterranean and aerial plant parts have been found to be affected by suboptimum temperature. Duke et al. (1979) reported that root weights for soybeans grown at root temperatures of 13^{o}C with an air temperature of 20^{o}C for 63 days were 12 percent of plants with root and air temperatures of 20^{o}C. The reduction in root growth was due to less branching and less differentiation. Roots appeared to be thick and fleshy. They also observed a much

lower leaf weight due to a reduction in leaf area and leaf number for the plants with roots of 13°C. Barlow et al. (1977) showed that the leaf elongation rate of corn seedlings responded rapidly to change in soil temperature. The elongation rate approached zero at root temperature of 12.5°C after declining steadily when the root temperature was lowered from 28°C. The restricted water uptake which lowered plant water potential and a decrease in temperature of the apical meristem was found to be responsible for the decrease in rate of leaf elongation. Rates of net photosynthesis and transpiration were not significantly affected until the soil temperature was lowered to 11.5°C.

MATERIALS AND METHODS

Preparation of Plant Materials

Soybean (Glycine max. (L.) Merr. cv. Wilkin) were inoculated with an inoculum containing the Rhizobium japonicum strain DES 122 and planted in germinating trays containing a mixture of moist peat and vermiculite. The trays were kept in a controlled temperature chamber set at day and night temperatures of 27 and 21°C respectively.

Recent alluvial sandy loam soil was taken from the stockpile in the greenhouse and sieved through a 2.5 mm screen. The soil was uniformly packed in 10 x 30 x 0.8 cm containers described by Sedgley and Boersma (1969). On the day after germination, seedlings were carefully transplanted into the soil in the lucite containers. Initial subsurface irrigation was achieved by immersing the containers in deionized water until saturation was reached throughout the soil by capillary action.

The seedlings were placed in a growth chamber maintained at 27 and 21°C day and night temperature, respectively, with 14 hours daylight provided by fluorescent and incandescent lamps irradiating about 400 $\mu E/m^2/sec$ in photosynthetic range at the plant canopy. The seedlings were irrigated on the fifth day after the initial watering with a modified Hoagland's solution (Appendix Table 1). The plants were watered every other day until about two weeks old, then daily irrigation was practiced to meet an increasing rate of transpiration. Salts accumulating at the soil surface were washed down the profile occasionally with deionized water.

Soil Water Potential and Soil Temperature Control System

The control of soil water potential was achieved by a system similar to that used in previous plant water studies in this laboratory (Sedgley and Boersma, 1969; Barlow and Boersma, 1976). Seamless dialysis tubing with a flat width of 11.70 cm was cut in sections to the required length. After folding the lower end and securing it with a plastic paper clip to prevent leakage, the tubing was soaked in distilled water for two hours. Covers from the lucite containers were removed, and the assembly was carefully slid into the semi-permeable membrane casing. The assemblies were then immersed in a mixture of nutrient solution and polyethylene glycol (Carbowax 6000) of known matric potential. The matric force is the major component of water potential for the polyethylene glycol (PEG) media (Steuter et al., 1981).

The concentration of PEG at the desired temperature was estimated using an empirical equation reported by Michel and Kaufman (1973),

$$\Psi_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.19 \times 10^{-7})C^2T \qquad (2)$$

where Ψ_s is the water potential of the PEG-6000 solution (bars), T is the temperature (^{o}C) and C is the concentration of PEG-6000 (g/kg H_2O). Equation (2) yields results similar to those obtained from a curve expressing the relationship between osmotic potential and concentration of PEG-6000 solution reported by Zur (1961). The semi-permeable membrane allows low molecular weight inorganic ions to move into the

soil and roots system, while excluding the high molecular weight PEG. When equilibrium is established, water in the soil will reach the same potential as that in the PEG and nutrient mixture.

Soil temperature was controlled by circulating water from a water bath through the outer compartment surrounding the osmotic chamber (Barlow and Boersma, 1976).

Radioactive Carbon Labeling System

A pulse labeling technique was chosen to estimate the rate of translocation from a soybean leaf at different environmental conditions. The advantage of this method was discussed earlier. The labeling systems described in the literature vary greatly due to specific measurements and experimental purposes. None of these was found suitable for use in association with the soil water potential and temperature control system employed in this study. It was necessary, therefore, to design an appropriate pulse labeling apparatus and an air flow control system in concert with the available facilities.

Radioactive Gas Generating Chamber

A chamber was made of 12.7 mm thick persplex with inside dimensions of 20 x 25 x 20 cm (Plate 1). The base had dimensions of 39 x 39 cm. A lid was clamped to the chamber by twelve wingnuts to provide an airtight seal using a neoprene gasket. A hole was made in the lid to accept a 5.0 cm long section of a 19.0 mm I.D. rigid persplex tube. The upper end of the tube was tightly sealed with a serum cap through which a hypodermic needle could be inserted for

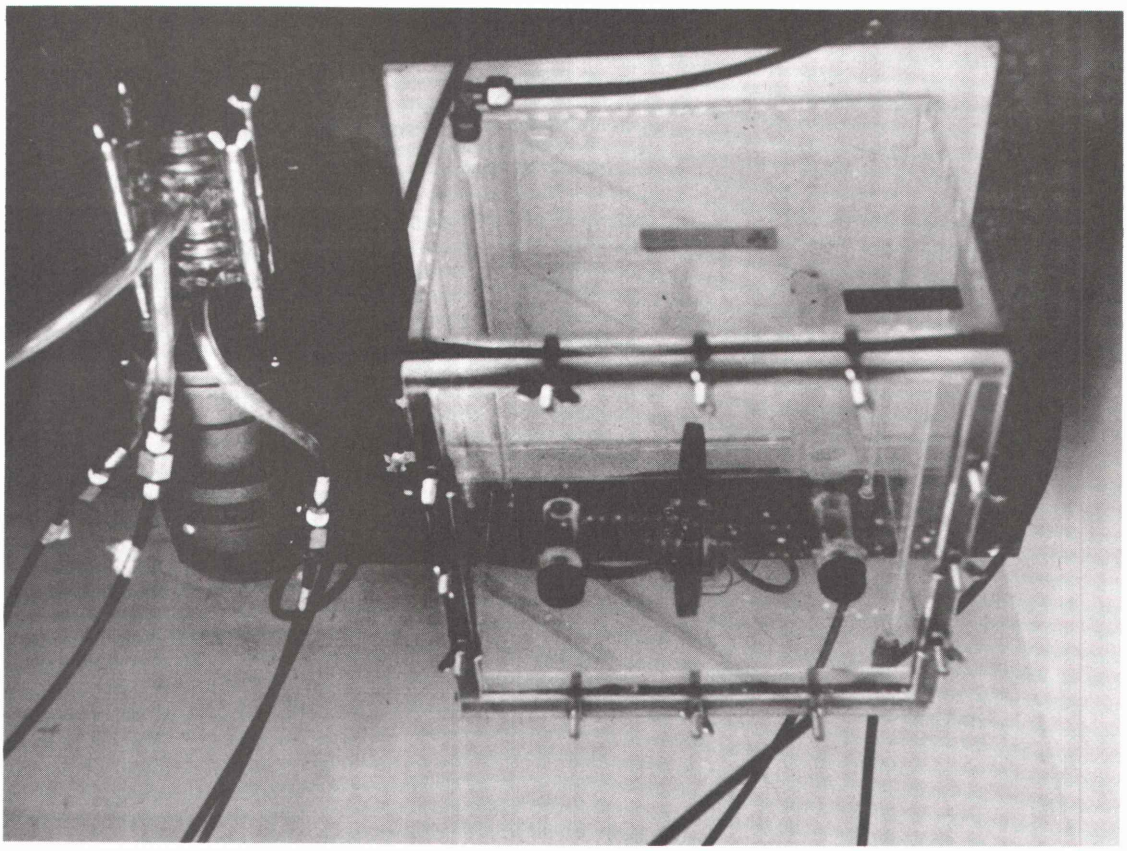

Plate 1. The $^{14}CO_2$ generating chamber and the peristaltic pump used in the labeling experiments.

delivering a 10 percent perchloric acid ($HClO_4$) solution to radioactive barium carbonate ($Ba^{14}CO_3$) contained in a beaker inside the chamber for generating $^{14}CO_2$.

Air could leave the chamber through small holes along a 6.4 mm diameter tube with ends attached to the side walls and the outlet connected to the feeding line with a Swagelog fitting outside the chamber. Air could enter the generating chamber from the leaf labeling chamber via a 6.4 mm O.D. inlet at one side of the wall (Plate 1).

Purging of the generating chamber at the termination of each labeling period was done by pumping air through the 6.4 mm O.D. inlet. A small electric fan was inside the chamber attached to a side wall in order to maintain uniform mixing of air inside the chamber. All inlet and outlet connections to feeding lines were equipped with gas-tight fittings. All parts were carefully checked for air leaks (Plate 1).

Chamber for Labeling the Leaves

The chamber for labeling leaves was made of persplex, 12.7 mm thick, with inside dimensions of 52.0 x 16.5 x 2.4 cm (Plate 2) and with water jackets on upper and lower sides for temperature control. The lower water jacket was cemented to the base of the chamber. The upper water jacket was a separate unit which was clamped to the chamber with eighteen wingnuts against a neoprene rubber gasket.

Air entered and left the chamber through a 6.4 mm O.D. inlet connected to a distribution tube of similar size inside the chamber. Rows of 0.4 mm diameter holes were drilled along the tube to distribute the air uniformly in the chamber. Three electric fans were

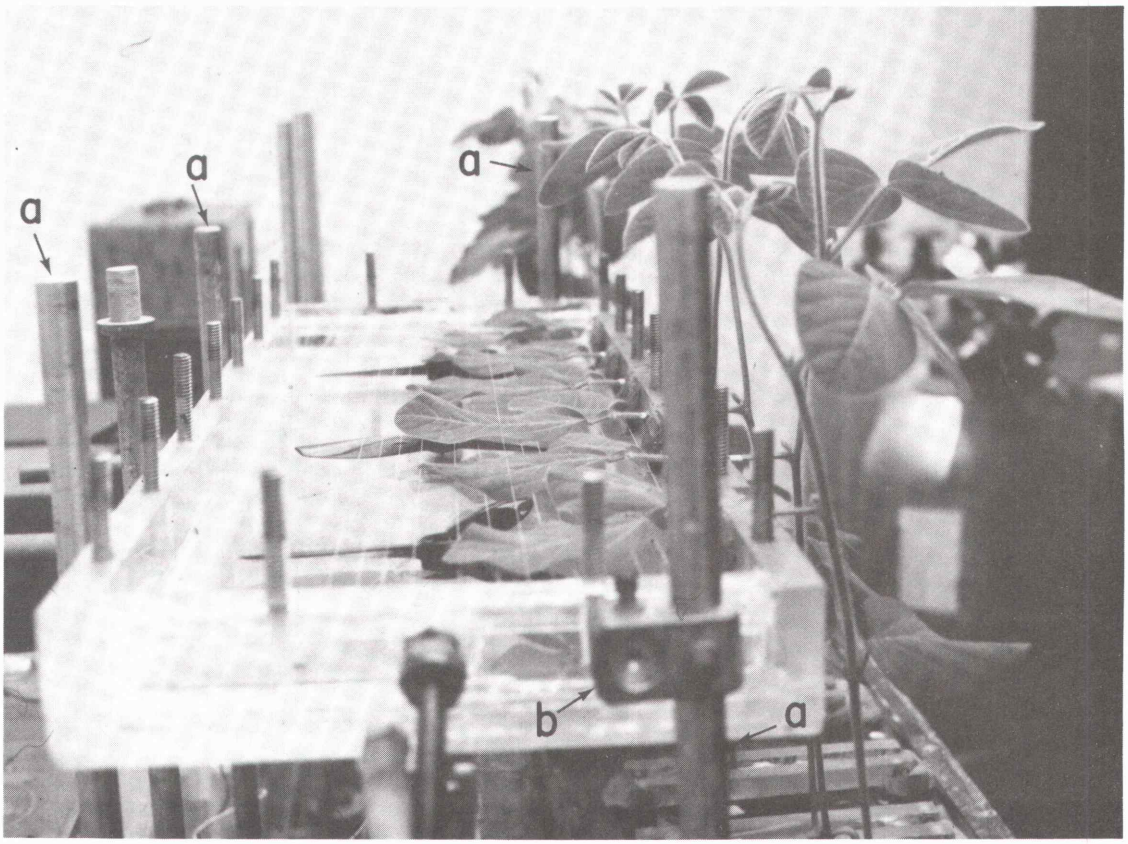

Plate 2. The leaf chamber with the upper compartment removed, showing six fully expanded first trifoliate leaves of soybeans in position for $^{14}CO_2$ labeling (a, supporting rods; b, right-angled rod clamp).

fixed to the base of the chamber such that only the fans emerged through the base (Plate 2). The motors were encased in persplex cylinders through the lower water jacket. Six slots, each 6 mm wide, were cut along one side of the wall to provide passage for the soybean petioles into the chamber for $^{14}CO_2$ labeling. Plasticine modeling clay was used for sealing along the petioles when leaves were in position.

The water jacket had inside dimensions of 52.0 x 16.5 x 1.0 cm with a 0.3 cm thick wall at the upper and the lower sides. Water maintained at a predetermined temperature was passed through the jackets. The water entered and left the water jacket through 6.4 mm O.D. tubes.

The labeling chamber was secured in place by putting clamps on the casings made of clear plastic of the fan motors below the chamber. The arms of the clamps were anchored to supporting rods (a) behind the osmotic chamber. Additional stability of the labeling chamber was achieved by attaching right-angled rod clamps (b) to the side walls of the chamber (Plate 2) with screws. The clamps were anchored by the aluminum supporting rods (a). This assembly made it possible to adjust the height of the chamber so that the height of the first trifoliate leaves to be labeled could be matched.

Control of $^{14}CO_2$ Flow System

Radioactive CO_2 was generated by injecting an excess of 10 percent perchloric acid from a syringe through a serum cap into a vial containing 50 microcuries of $Ba^{14}CO_3$ with specific activities of 5.0 millicuries per millimole in the generating chamber (G, Plate 3).

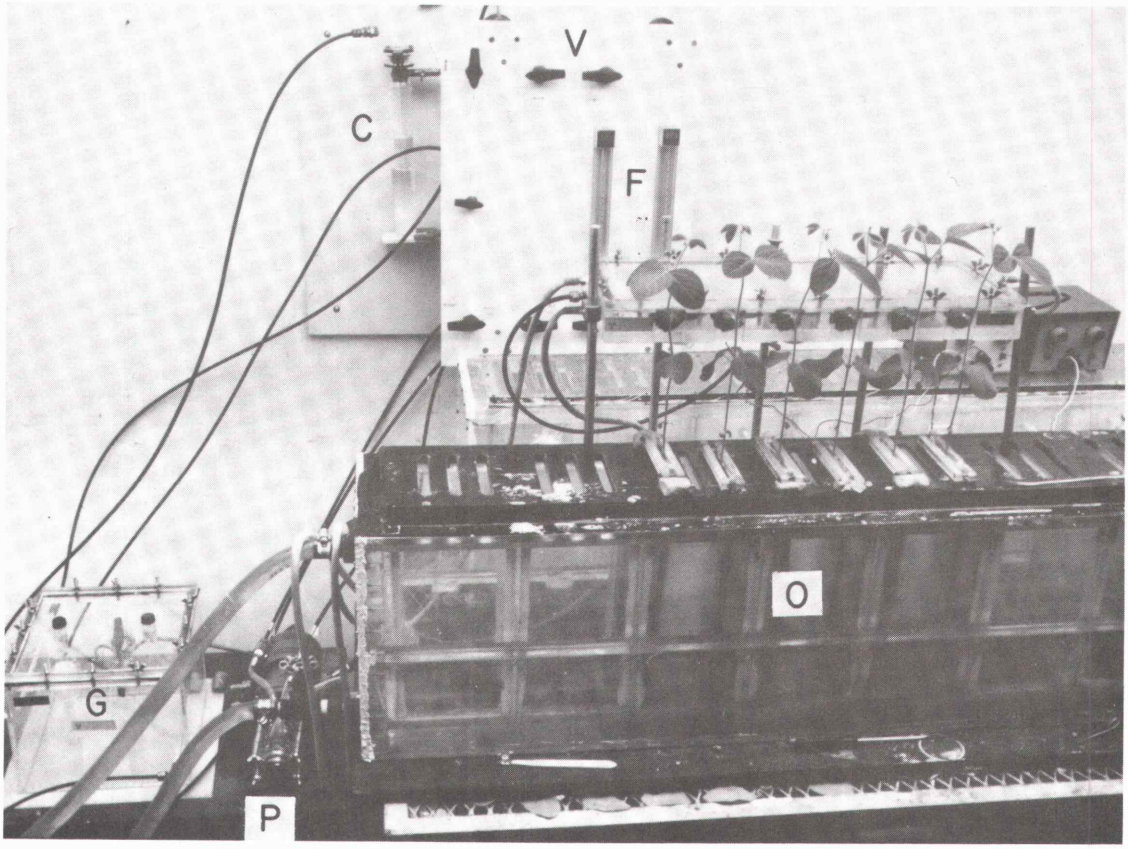

Plate 3. The arrangement of the equipment used in the $^{14}CO_2$ labeling experiment showing G, $^{14}CO_2$ generating chamber; P, peristaltic pump; O, osmotic chamber; L, leaf labeling chamber; F, flow meters; V, three-way valves; and C, NaOH column.

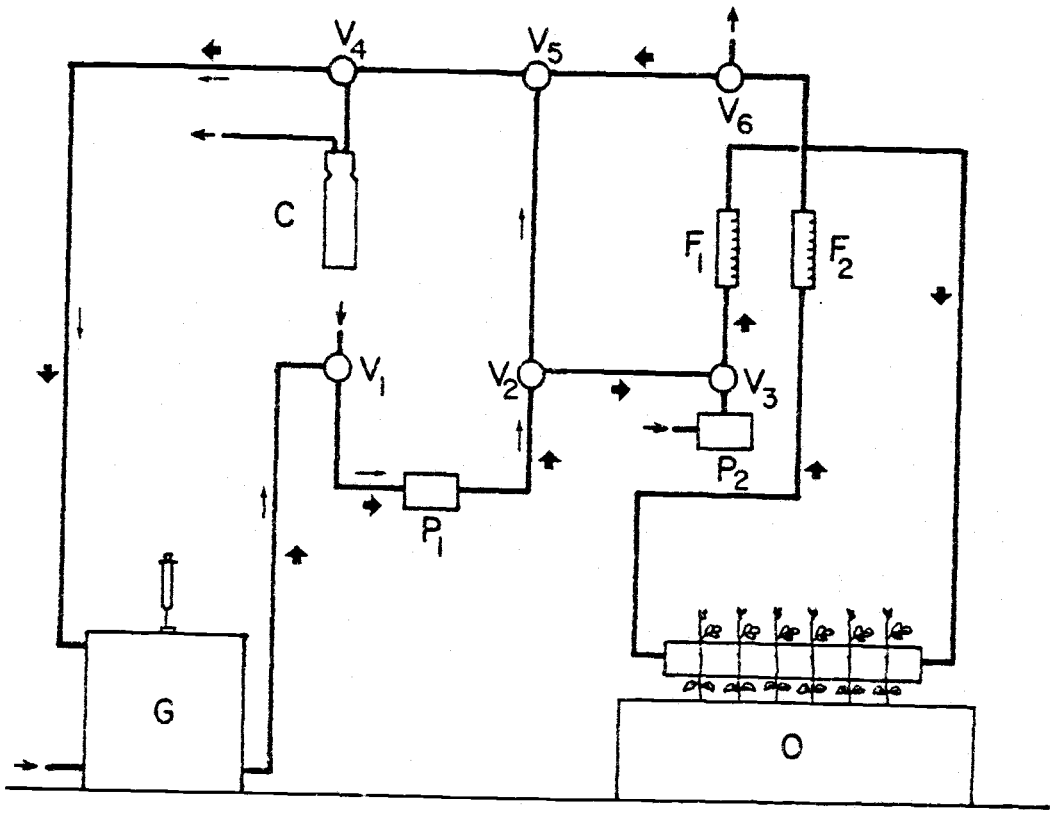

Figure 1. Schematic design of $^{14}CO_2$ flow control system.

The amount of $^{14}CO_2$ generated from the reaction was 10 μmoles. The labeled $^{14}CO_2$ released from the reaction was drawn from the generating chamber to the 20 liter bottle air mixing reservoir positioned between the generating chamber and peristaltic pump P_1. The flow was directed along the closed loop indicated with thin arrows in Figure 1, by adjusting three-way valves V_1, V_2, V_4, and V_5. The position of air mixing reservoir is not shown in Figure 1. Circulation was continued for 45 minutes with peristaltic pump P_1 to obtain uniform mixing of air and $^{14}CO_2$ before labeling. The concentration of $^{14}CO_2$ in the system was about 8.0 ppm by volume. Labeling of the leaves was initiated by adjusting valves V_1, V_2, V_3, V_4, V_5 and V_6 so that $^{14}CO_2$ was supplied to the leaf chamber along the path indicated by the thick arrows in Figure 1. After a 5 minute labeling period, the leaf chamber was purged with the peristaltic pump P_2 and excess radioactive gas was trapped in a NaOH column (C, Plate 3) positioned near the regulating panel. The flow meters F_1 and F_2 were used to monitor the flow rate of air with the $^{14}CO_2$ into and out of the labeling chamber, respectively. Gas leaks along the leaf chamber were initially searched for by looking for significant differences in the readings between inflow and outflow rate. Leaks were pinpointed with soapy water and sealed. Excess radioactive gas was purged from the generating chamber by pumping air through the air inlet at one of the side walls of the generating chamber. This air was directed through the chamber to the NaOH column by regulating valves V_1, V_2, V_4, V_5 and pump P_1. Escaped $^{14}CO_2$ from the NaOH column, if any, was guided to a fume hood outside the controlled temperature room.

Experimental Procedure for $^{14}CO_2$ Labeling Studies

Two days before an experimental run commenced, six of the 18-day old seedlings with first trifoliate leaves fully expanded and petioles at similar height were selected. In this discussion the oldest leaf is designated as the first leaf, the next oldest leaf is the second leaf and so on. Side walls of the lucite boxes containing the individual seedlings were removed, the soil slabs were encased in semi-permeable membranes, and immersed in osmotic chambers containing the PEG-6000 and nutrient solution at a specified water potentials. The temperature of the water circulating around the osmotic chambers was preset at the desired level prior to transferring the plants. The plants were allowed to equilibrate with the ambient environment in continuous light at 400 $\mu E/m^2/sec$ at the top of the canopy from fluorescent and incandescent lamps in the controlled temperature room with air temperature at $27^{\circ}C$ and relative humidity at 50 ± 5 percent for two days before the start of the labeling experiment.

Two hours before $^{14}CO_2$ labeling started, one of the side leaflets of the first trifoliate set of leaves of each plant was excised. The cut surface was immediately sealed with a mixture of lanolin and beeswax to prevent loss of water. The remaining two leaflets were placed inside the labeling chamber. Trimming the leaves of plants has been practiced in many translocation studies and did not seem to alter translocation pattern of photosynthates (Tully and Hanson, 1979; Geiger and Fondy, 1979; Housely et al., 1977). The petiole was wrapped with teflon tape and carefully placed on modeling clay in the groove in the side wall of the leaf chamber. The purpose of the teflon tape was to avoid direct contact between petiole tissues and modeling clay.

Chemicals from the clay were found to cause "burning" on the petiole tissue and translocation from the leaves seemed to be affected in test experiments. The height of the chamber was adjusted to the height of the petioles of the plants using supporting clamps. Small electric fans inside the chamber were turned on after leaves were positioned properly. The upper compartment of the chamber was then closed by putting it in place and tightening the wingnuts and water at 27°C was circulated through the water jackets. The system was checked for air leakage using soapy water and allowed to equilibrate for two hours. During the equilibration period $^{14}CO_2$ was generated in the generating chamber and the air containing the radioactive gas was circulated in the closed loop for 45 minutes before labeling commenced as previously explained.

Labeling was initiated by directing the air containing $^{14}CO_2$ into the leaf chamber at the rate of 1.5 l min^{-1}. The calculated velocity of air flow across the chamber was about 0.006 m sec^{-1} but the actual velocity was greater than this value because of the air turbulence generated by the electric fans inside the chamber. After a 5 minute labeling period, the atmosphere in the leaf chamber was purged with air for 5 minutes before leaves were removed from the chamber. Plants were then allowed to photosynthesize in the controlled temperature room environment. Plants were sampled randomly later at 1, 2, 4, 8, 12 and 24 hours for translocation measurements.

Each plant was cut into sections as follows: (a) source leaf (the labeled first trifoliate leaf and the 1 cm long portion of the petiole inside the labeling chamber), (b) primary leaf, (c) second trifoliate leaf, (d) tip (the growing tip of the plant above the

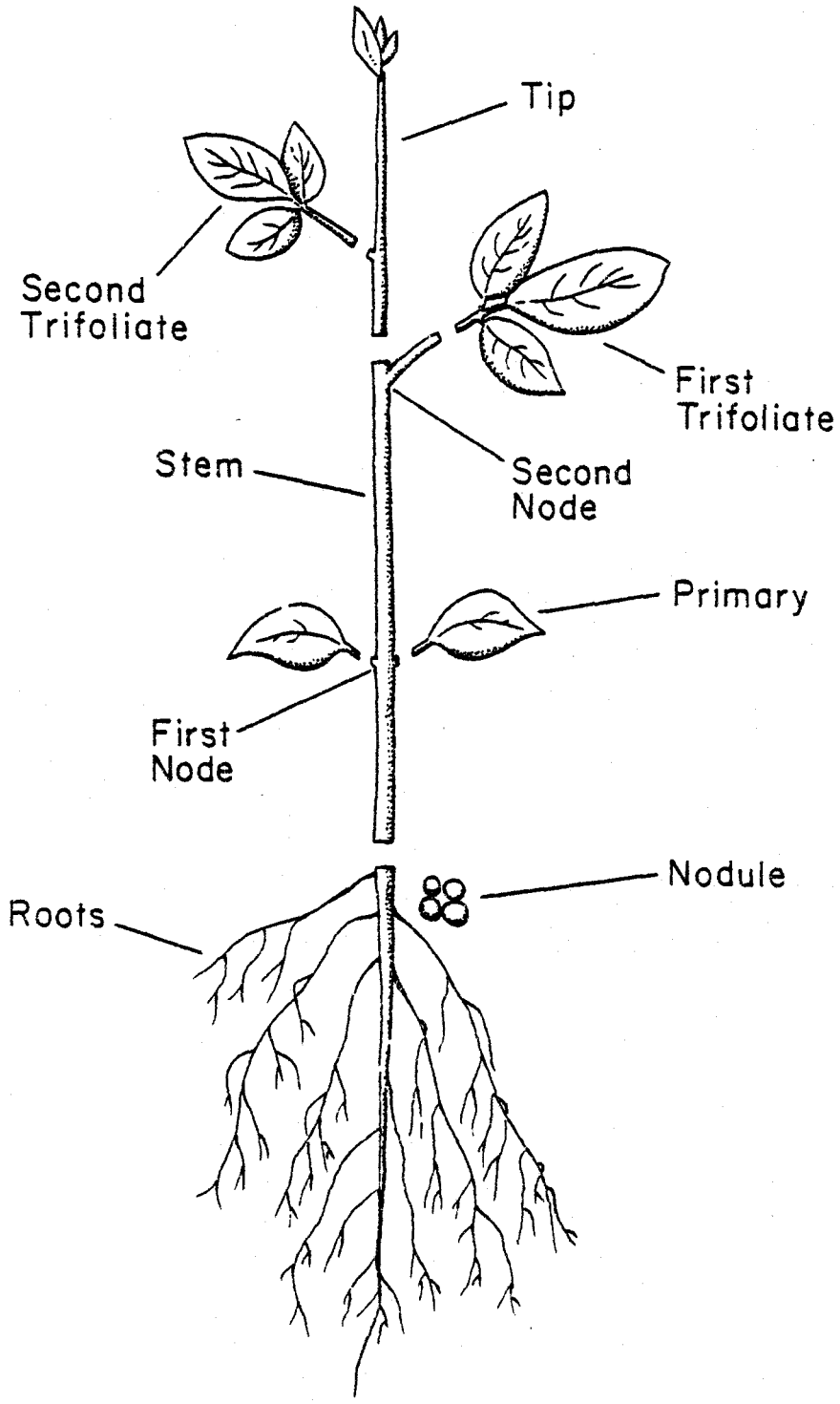

Figure 2. Diagram showing the plant parts used in the analysis of labeling experiments.

second foliar node including young expanding third and fourth trifoliate leaves, (e) stem (stem from the soil surface to the second foliar node and about 1 cm of the petiole of the first trifoliate leaf), (f) roots and (g) nodules. Figure 2 illustrates these sections.

Each plant part was dried separately at 75°C in an oven for 48 hours. The dried materials were carefully ground with mortar and pestle, and combusted in a Packard Model 306 Sample Oxidizer. The recovered $^{14}CO_2$ was trapped in an organic base and fluor mixture. Counting of radioactivities in each sample was done on a Beckman Model LS 7500 liquid scintillation counter. The activities were expressed as disintegrations per minute (dpm) and dpm per mg tissue.

The labeling experiments were arranged in a split-split-plot design with soil temperatures at 25°C and 10°C as main plots, soil water potentials at -0.35, -2.5 and -5.0 bars as sub-plots, and six different sampling times after the labeling period as sub-sub plots. Three replications of each treatment were obtained.

Measurements of Water Potential and Growth

Nomenclature

To facilitate the discussion, a number of symbols are used throughout the rest of this report. They are listed as follows,

B	apoplastic water fraction,
N_s	number of moles of dissolved solutes in the plant cells,
P	balance pressure corresponding to a certain volume of sap expressed in the P-V measurements,
R	gas constant,
RWC	relative water content,

RSWC	relative symplastic water content,
T	Kelvin temperature,
V_e	volume of sap expressed by an increment increase in balance pressure in the P-V measurements,
V_s	symplastic volume of the tissue,
V_o	symplastic volume of the tissue at full turgor,
V_T	total water volume of the tissue, the sum of apoplastic and symplastic water volume,
k	turgor potential at which $\varepsilon/\varepsilon_m$ is equal to $1 - 1/e$,
α	factor for correcting the value of osmotic potential measured by a dew point hygrometer,
ε	bulk volumetric elastic modulus,
ε_m	maximum value of bulk volumetric elastic modulus,
π	osmotic potential of tissue sap,
π_o	osmotic potential of the leaf tissue at full turgor,
π_p	osmotic potential at incipient plasmolysis or zero turgor potential.

Leaf Water Potential

The pressure chamber technique (Scholander, 1964) was used for measuring leaf water potential. The petiole of soybean seedlings is not rigid and it has a groove along its length which presents difficulties in preventing air leakage at the interface between the petiole and the rubber stopper normally used with PMS-type sample holders. To solve this problem, a rapid-setting RTV41 silicone rubber compound (G.E. Co.) and RTV catalyst type F (Dow-Corning Co.) were used to prepare a molded sample holder. The mixture was poured into a plastic container with a soybean petiole, similar in size to

the one to be used in the experiment, held in place at the center of the container. The plastic container had the same size and shape as the rubber stopper normally used with the sample holder. The silicone compound was allowed to settle and cure at room temperature for at least two hours. The petiole was then carefully pulled from the cured silicone. This left a hole at the center of the silicone stopper in the shape of the petiole. The height of the stopper was adjusted by cutting to fit the sample holder. The silicone stopper was found to be softer and more elastic than the normally used rubber stopper. It provided a better seal.

To minimize leaf dessication during the measurements, the trifoliate leaves were quickly wrapped with two layers of small plastic bags after cutting (Wenkert et al., 1978c; Wilson et al., 1979). The string was tied loosely around the bags at the petiole. For measurement, the petiole was guided through the hole in the silicone stopper with a piece of copper tubing. The orientation of the petiole was arranged to match the shape of the soybean petiole left by molding. Sample preparation took about one minute.

The pressure inside the chamber was slowly increased at the rate of about 0.1 bar s^{-1} with a slower rate near the anticipated end point. Often, false end points were experienced with gas bubbles emerging from one or more vascular bundles. If the pressure was reduced about 1.5 bars and sap was not withdrawn into the vascular bundle, the true end point was considered not to have been reached and the sap was removed with cotton wool. The pressure was then increased again until the true end point was observed and the pressure reading was noted.

The depressurization and pressurization was repeated again to confirm the pressure reading at the end point.

Osmotic Potential Measurements

The trifoliate leaves taken from the pressure chamber following the leaf water potential determination were rolled into a cylinder which was inserted into a piece of tygon tubing. The sample in the tubing was quickly frozen in dry ice. At the time of measurement the sample was thawed for at least one hour. Cell sap was expressed by first rolling a block of wood over the tygon tubing several times to rupture the cell walls and to obtain mixing of symplastic and apoplastic water. The tubing was then squeezed between two steel rollers, made for this purpose, for further mixing of the sap and to squeeze the sap to the upper end of the tubing. A filter paper disc with a diameter of 7 mm was used to absorb some of the sap. The disc was placed in a shallow sample holder for osmotic potential measurements.

The measurements of osmotic potential of the cell sap were made with the Wescor HR-33T microvoltmeter and C-52 sample holder. Prior to the measurement, the cooling coefficient (π_v) of the hygrometer was set for the ambient temperature. Microvoltmeter readings were calibrated against five known osmotic standards made up of KCl solutions before each series of measurements. The measurements were made in the controlled temperature room at 25.0°C. Drifting of the microvoltmeter was observed when the temperature changed more than 5°C during the day in the laboratory. Contamination of the sample holder and the thermocouple was always checked before measurements. Cleaning

of the contaminated surface was achieved by delivering a few drops of reagent grade acetone with a syringe to the depression of the thermocouple mount. The liquid was shaken off and then droplets of double distilled water were applied to the mount. The application and removal of water droplets was repeated several times. Remaining water droplets were blown away by short bursts of dry air from a pressurized dry air can (Dust-Off, Falcon Safety Products, Inc.). Care was taken to hold the can in an upright position.

Tissue osmotic potential obtained by the above method may not represent the true osmotic potential of the free solution in cytoplasm and vacuole, because thawing and crushing of the tissue permits mixing of cell wall water with symplastic solutes. Thus the osmotic potential is decreased and a negative turgor pressure may be calculated as explained by Tyree (1976). Correcting this error is possible by obtaining the apoplastic water fraction (B), the relative water content of the leaf tissue (RWC).

The correction factor (α) is calculated from,

$$\alpha = \frac{RWC}{RWC - B} \qquad (3)$$

The corrected value of osmotic potential is then obtained by multiplying the value of osmotic potential measured with a dew point hygrometer by the correction factor.

Determination of the P-V Curve

A procedure to obtain P-V curves was introduced by Scholander et al. (1964) and has been used to characterize plant water relations in woody species (Cheung et al., 1975); soybeans (Wenkert et al., 1978);

rice (Cutler et al., 1979); and corn (Wenkert, 1980). A pressure chamber is used to obtain paired measurements of leaf water potential, and water content of the leaf for a succession of decreasing tissue water contents (Figs. 3 through 6). From these measurements a curve of potential vs. water content can be constructed. Values of osmotic potential and turgor potential can be calculated using this curve. The data also allow the determination of elastic modulus of the tissue at different water content, symplastic water volume, and apoplastic water fraction.

The theory relating the change in balance pressure to the volume of water expressed by the change was developed by Tyree and Hammel (1972) and later simplified by Tyree (1976). One of the assumptions is that the turgor pressure, once it reaches zero, remains at zero and that the matric potential of the symplastic water is negligible.

Osmotic potential (π) is related to number of moles of dissolved solute in the plant cells (N_s) and symplastic water volume (V_s) according to the van't Hoff equation,

$$\pi = \frac{-RT}{V_s} \cdot N_s \tag{4}$$

where R is the gas constant and T the Kelvin temperature. Since leaf water potential (Ψ) is the sum of osmotic potential (π) and turgor potential (Ψ_p), $\Psi = \pi$ when Ψ_p falls to zero, during the procedure so that

$$\frac{1}{\Psi} = \frac{-V_s}{N_s RT} \tag{5}$$

or

$$-\frac{1}{\Psi} = \frac{V_o}{N_s RT} - \frac{V_e}{N_s RT} \tag{6}$$

and

$$\frac{-1}{\pi} = \frac{V_o}{N_s RT} - \frac{V_e}{N_s RT} \qquad (7)$$

where V_o is the symplastic volume at full turgor and V_e is the volume of expressed sap. Equation (6) predicts a linear relationship between $(-1/\Psi)$ and V_e over the range where $\Psi_p = 0$.

Figures 3 through 6 show the plots of $-1/\Psi$ against V_e. For the linear part of the plots the osmotic potential can be calculated with Equation (7).

The turgor potential (Ψ_p) can be obtained from the P-V measurement as the difference between Ψ and π. This calculation assumes that the amount of solutes remains constant during the extraction procedure.

Symplastic Water Volume at Full Turgor (V_o)

The symplastic water volume at full turgor includes all water inside the living cells. The value of V_o is determined by extrapolating the straight line to infinite balance pressure (P) or at $1/P = 0$ (Fig. 3).

Osmotic Potential at Full Turgor (π_o)

The value of π_o is determined by the amount of solutes a leaf contains per unit volume of symplastic water, at zero leaf water potential. The reciprocal of π_o can be obtained by extrapolation of the straight line segment of the P-V curve to the ordinate (Fig. 3).

Osmotic Potential at Incipient Plasmolysis (π_p)

The osmotic potential of the leaf tissue at zero turgor potential is the osmotic potential at the inflection on the P-V curve (Fig. 3).

Relative Water Content (RWC)

The relative water content of the tissue is calculated from

$$RWC = \frac{V_T - V_e}{V_T} \times 100 \qquad (8)$$

where V_T is the total water volume of the tissue including symplastic and apoplastic water, and V_e is the volume of expressed sap.

Relative Symplastic Water Content (RSWC)

The relative symplastic water content of the tissue is defined as,

$$RSWC = \frac{V_o - V_e}{V_o} \times 100 \qquad (9)$$

where V_o is the symplastic volume at full turgor.

Bulk Volumetric Elastic Modulus (ε)

The bulk volumetric elastic modulus of the tissue is defined by Dainty (1976) as,

$$\varepsilon = \frac{d\Psi_p}{dV_s} \cdot V_o \qquad (10)$$

From Equation (9) and from $V_s = V_o - V_e$, Equation (10) becomes,

$$\varepsilon = \frac{\Delta\Psi_p}{\Delta RSWC} \qquad (11)$$

Apoplastic (Bound) Water Fraction (B)

The apoplastic water fraction is the portion of water in the xylem and cell walls. It can be expressed as,

$$B = \frac{V_T - V_o}{V_T} \tag{12}$$

Procedure for Constructing the P-V Curve

Twelve hours before P-V measurements were made, the petiole of the leaf to be used was cut under distilled water 2 cm from the base of the terminal leaflet. The petiole was kept submerged in distilled water in a small beaker with leaflets above the water surface. The beaker with the leaf samples was placed in a large plastic bag. The atmosphere inside the bag was humidified by spraying with a fine mist before tightening the open end of the bag with a rubber band. Air inside the bag maintained sufficient pressure on the walls to support them and maintain the shape.

At the beginning of the experiment, the leaf sample was removed from the humidified plastic bag. Water remaining on the surface of the petiole was wiped off with cotton wool. The leaf was then wrapped with two layers of small plastic bags and quickly positioned inside the pressure chamber. The inside walls of the chamber were lined with wet filter paper in order to minimize evaporation and temperature fluctuations during the measurement.

The initial leaf water potential was determined according to the procedure previously outlined. After reading the balance pressure, a pre-weighed sap collector, made of 3.0 mm inside diameter tygon tubing, packed with cotton wool with one end open and one end closed with a

rubber stopper was placed over the cut end of the petiole. The chamber was slowly pressurized at the rate of 2 bars min^{-1} to a pressure of about 2 to 10 bars above the previous balance pressure. The elevated pressure was maintained for a period of about 2 minutes at the beginning of each P-V measurement. The exchange times were increased up to 30 minutes at the end of the series of P-V measurements. The appropriate overpressure and exchange times were determined empirically and depend on plant species (Tyree et al., 1978; Cutler et al., 1979). The appropriate combinations will give enough data points for plotting a reliable P-V curve.

During each pressure increment expressed sap was absorbed by the collector. After the equilibration period at the end of each pressure step the sap collector was removed and weighed with accuracy of $\pm$ 0.1 mg. The amount of sap expressed obtained as the difference between initial and final weight was recorded. The pressure was lowered to a pressure between the previous balance and the overpressure. Time was allowed for the sap to be withdrawn into the vascular bundles and equilibrated with the pressure inside the pressure chamber. The new balance pressure was determined from the applied pressure at which the sap reappeared at the cut surface of the petiole. The procedure was repeated for the number of measurement pairs required for plotting the P-V curve. Sufficient data points are needed for extrapolation of the linear portion.

At the end of the series of measurements, residual fresh weight of the leaf was determined before drying it in an oven at 75°C for 48 hours. Oven dry weight was also obtained. The difference between residual fresh weight and dry weight was added to the weight of the

total amount of water expressed from the leaf to obtain the original total water volume.

Paired measurement data were plotted with the inverted balance pressure (1/P) on the ordinate and the expressed sap volume (V_e) on the abscissa (Figs. 3 through 6). Paired data for the linear portion of each P-V curve were analyzed using linear regression analysis. Values characterizing internal plant water relations were calculated and are tabulated in Tables 1 through 4.

Experimental Procedure for Plant Growth and Water Relation Studies

Twenty-four soybean seedlings similar to the ones used in the labeling experiments were selected, their roots and soil systems were encased in the semipermeable membranes and the assemblies were immersed in the solutions of PEG-6000 and nutrients at the same environmental conditions as used in the labeling experiments. Seedlings were randomly assigned to each soil water potential level of -0.35, -2.5 and -5.0 bars. Soil temperature treatments were 25 and 10°C. After a 48 hour equilibration period in continuous light, the length of the first, second, third, and fourth trifoliate leaves along the petioles and midveins were measured. Measurements were repeated 24 hours later on the same leaves. The differences were taken as elongation rates at each level of treatment.

Seventy-two hours after the plants were transferred to the osmotic chambers, the fully expanded first trifoliate leaf of the seedling in each water potential treatment was excised and quickly put in the pressure chamber for the leaf water potential measurement. It was subsequently frozen in dry ice for determination

of osmotic potential. The experiments were arranged in a split-plot design with four replications, with soil temperature at 25 and 10°C as main plots, and soil water potentials at -0.35, -2.5, and -5.0 bars as the subplots.

RESULTS AND DISCUSSION

Characterization of Plant Characteristics by Pressure-Volume Measurements

P-V Curves

Pressure volume curves were obtained according to procedures described above (Figs. 3 through 6). The plots have the typical characteristics of P-V curves observed by many investigators (Tyree, 1972; Cutler et al., 1979; Wilson et al., 1979). Regression analysis of the straight line portion yielded r^2 values for the four leaf samples ranging from 0.98 to 0.99. These regression equations are shown in Figures 3 through 6 for each of the leaf samples. The equations were used for estimating $1/\pi$ at corresponding values of ΣV_e as shown in Tables 1 through 4.

Relationships between leaf fresh weight and balance pressures or leaf water potentials are shown in Figure 7 for all four leaves. Leaf fresh weights were obtained by adding residual water and sap volumes expressed to the leaf dry weight. The first three or four readings were used for extrapolation to zero water potential (Fig. 8) to obtain the leaf fresh weight, hence leaf water content, at full turgor (Ladiges, 1975).

Analysis of the P-V data (Tables 1 through 4) showed that the mean value of osmotic potential at full turgor was -9.61 ± 0.33 bars. This value is within the range reported by other workers for other herbaceous species (Jones and Turner, 1980; Wilson et al., 1979). The mean value of osmotic potential at zero turgor potential was -13.61 ± 1.18 bars. Zero turgor potential corresponded to mean leaf water potential of 13.72 $\pm$ 1.22 bars and a mean relative water content of 81.26 $\pm$ 6.24 percent.

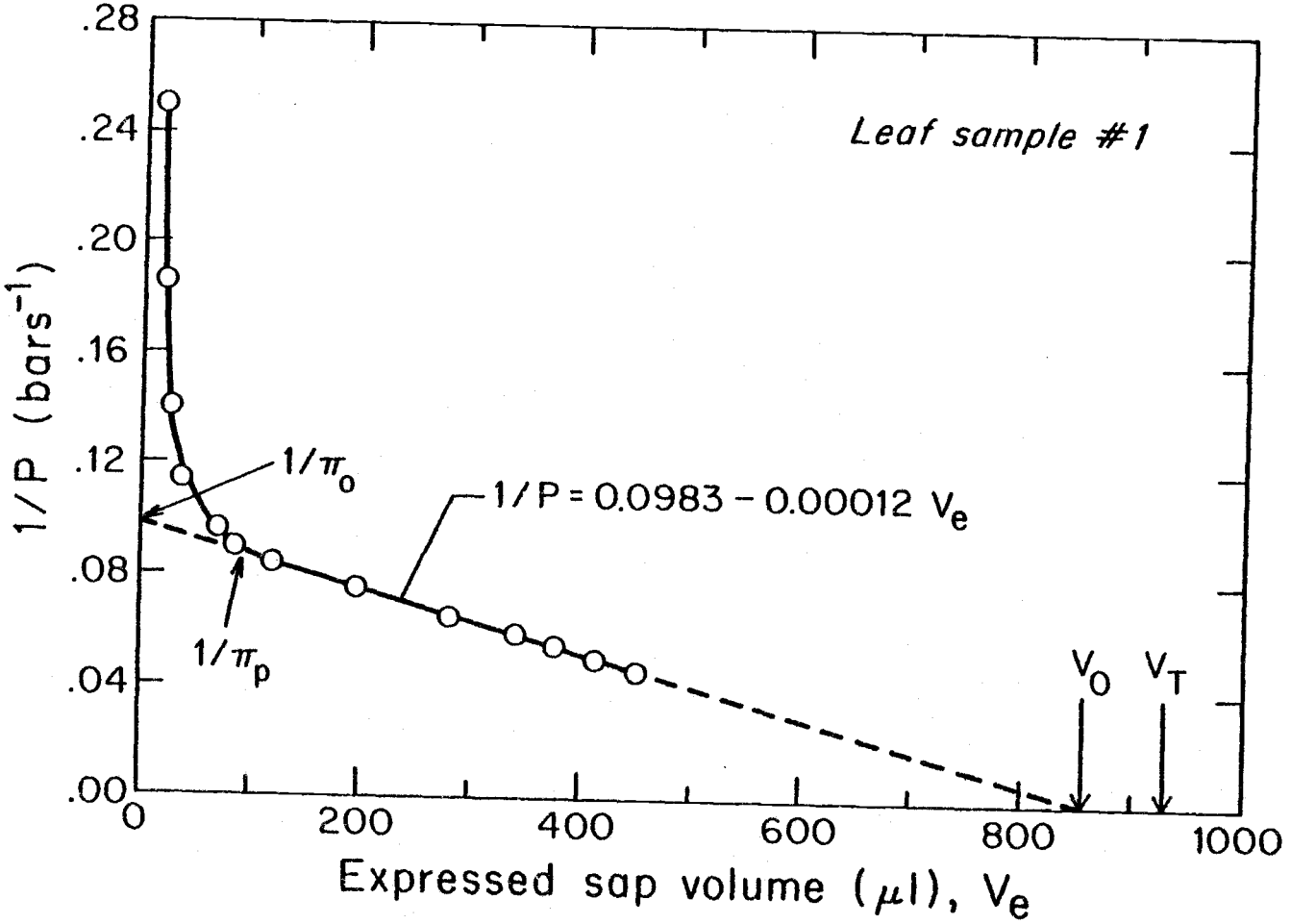

Figure 3. Water release curve of fully expanded trifoliate leaf (Sample No. 1).

Table 1. Data obtained for construction of pressure-volume relationship of soybean leaf sample No. 1.

Balanced pressure (P)	$\frac{1}{P}$	Leaf weight	Sap expressed V_e	RWC	ΣV_e	$-\frac{1}{\pi}$	$-\pi$	Ψ_p	$\Delta\Psi_p$	RSWC	ΔRSWC	Elastic modulus ε
bar	bar^{-1}	mg	µl	%	µl	bar^{-1}	---------bar----------			------%-------		bar
0	∞	1148.5*	0	100.00	0	0.0983	10.18	10.18	-	100.00	-	-
1.72	0.5814	1141.5*	7.0*	99.24	7.0	0.0974	10.26	8.54	1.64	99.18	0.82	200.00
3.96	0.2525	1131.6	9.9*	98.16	16.9	0.0963	10.38	6.42	2.12	98.02	1.16	182.76
5.38	0.1859	1126.7	4.9	97.63	21.8	0.0957	10.44	5.06	1.36	97.44	0.58	234.48
7.07	0.1414	1119.1	7.6	96.80	29.4	0.0949	10.54	3.47	1.59	96.55	0.89	178.68
8.76	0.1142	1109.0	10.1	95.70	39.5	0.0937	10.67	1.91	1.56	95.36	1.19	131.09
10.41	0.0961	1075.5	33.5	92.05	73.0	0.0898	11.13	0.72	1.19	91.43	3.93	30.28
11.03	0.0907	1063.1	12.4	90.70	85.4	0.0884	11.31	0.28	0.44	89.97	1.46	30.14
11.86	0.0843	1027.3	35.8	86.80	121.2	0.0843	11.87	0.01	0.27	85.77	4.20	6.43
13.24	0.0755	950.8	76.5	78.46	197.7	0.0754	13.25	0.01	0.00	76.78	8.99	0.00
15.38	0.0650	864.9	85.9	69.11	283.6	0.0655	15.26	0.20	-	66.70	10.08	-
16.90	0.0592	806.8	58.1	62.78	341.7	0.0588	17.00	-	-	59.88	6.88	-
18.07	0.0553	771.7	35.1	58.95	376.8	0.0548	18.25	-	-	55.75	4.13	-
20.00	0.0500	733.4	38.3	54.78	415.1	0.0504	19.86	-	-	51.26	4.49	-
21.72	0.0460	696.3	37.1	50.74	452.2	0.0461	21.70	-	-	46.90	4.36	-

*Extrapolation from Figure 8.

Dry weight = 230.5 mg

Residual water = 465.8 mg

Bound water = 7.24%

π = osmotic potential

Ψ_p = turgor potential

RWC = relative water content

RSWC = relative symplasmic water content

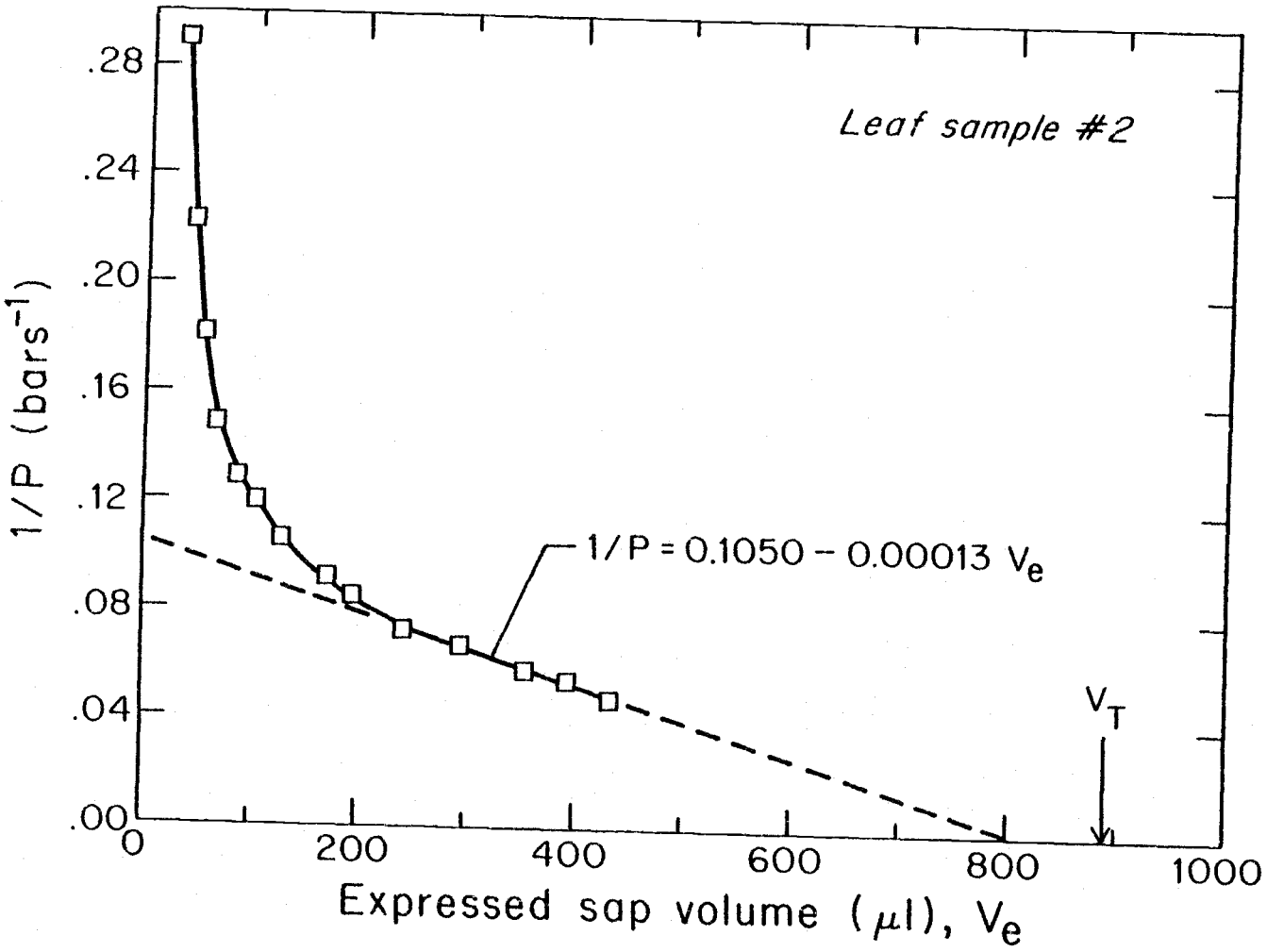

Figure 4. Water release curve of fully expanded first trifoliate leaf (Sample No. 2).

Table 2. Data obtained for construction of pressure-volume relationship of soybean leaf sample No. 2.

Balanced pressure (P)	$\frac{1}{P}$	Leaf weight	Sap expressed V_e	RWC	ΣV_e	$-\frac{1}{\pi}$	$-\pi$	Ψ_p	$\Delta\Psi_p$	RSWC	ΔRSWC	Elastic modulus ε
bar	bar^{-1}	mg	µl	%	µl	bar^{-1}	---------bar---------			------%------		bar
0	∞	1135.0*	0	100.00	0	0.1050	9.52	9.52	-	100.00	-	-
2.70	0.3704	1109.2	24.8*	97.21	24.8	0.1015	9.85	7.15	2.37	96.90	3.10	76.45
3.45	0.2899	1100.4	8.8	96.22	33.6	0.1003	9.97	6.52	0.63	95.80	1.10	57.27
4.48	0.2232	1090.6	9.8	95.12	43.4	0.0991	10.10	5.62	0.90	94.58	1.22	73.77
5.52	0.1812	1080.0	10.6	93.93	54.0	0.0977	10.24	4.72	0.90	93.25	1.33	67.67
6.72	0.1488	1066.1	13.6	92.40	67.6	0.0959	10.43	3.71	1.01	91.55	1.70	59.41
7.76	0.1289	1047.9	18.5	90.32	86.1	0.0935	10.70	2.94	0.77	89.24	2.31	33.33
8.33	0.1200	1028.7	19.2	88.16	105.3	0.9010	10.99	2.66	0.28	86.84	2.40	11.67
9.52	0.1050	1994.1	34.6	85.39	129.9	0.0864	11.57	2.05	0.76	82.51	4.33	17.55
10.87	0.0920	962.0	32.1	80.66	172.0	0.0822	12.16	1.29	0.76	78.50	4.01	18.95
11.76	0.0850	937.5	24.5	77.90	196.5	0.0790	12.66	0.90	0.39	75.44	3.06	12.75
13.88	0.0720	890.5	47.0	72.62	243.5	0.0729	13.73	0.00	0.90	69.56	5.90	15.25
14.92	0.0670	838.1	52.4	66.72	295.9	0.0660	15.15	-	-	63.01	6.55	-
17.24	0.0580	777.8	60.3	59.94	356.2	0.0581	17.21	-	-	55.48	7.53	-
18.52	0.0540	740.1	37.7	55.70	393.9	0.0532	18.81	-	-	50.76	4.72	-
21.28	0.0470	699.6	40.5	51.15	434.4	0.479	20.89	-	-	45.76	5.06	-

*Extrapolation from Figure 8.

Dry weight = 254.8 mg

Residual water = 454.8 mg

Bound water = 10.03%

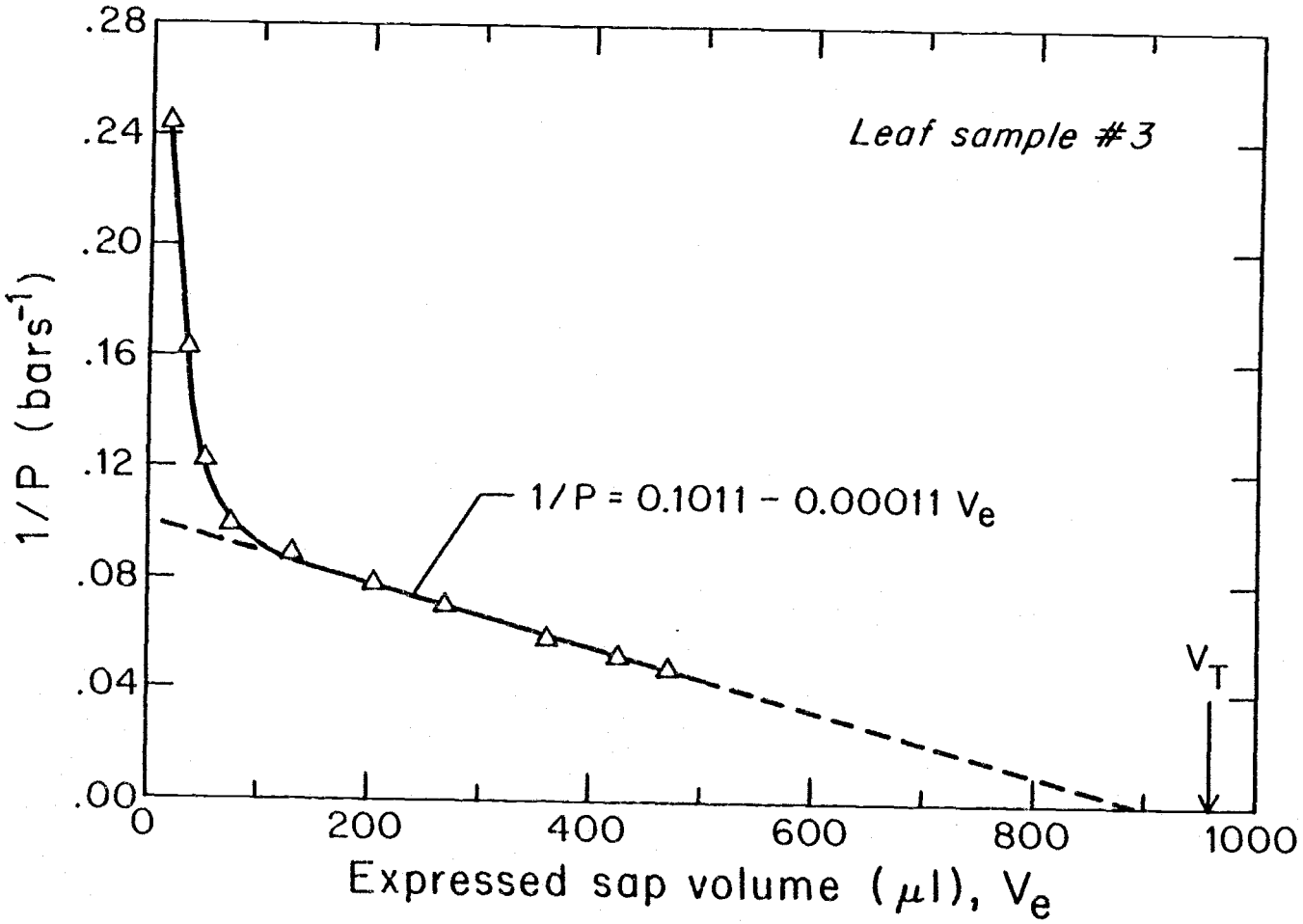

Figure 5. Water release curve of fully expanded first trifoliate leaf (Sample No. 3).

Table 3. Data obtained for construction of pressure-volume relationship of soybean leaf sample No. 3.

Balanced pressure (P)	$\frac{1}{P}$	Leaf weight	Sap expressed V_e	RWC	ΣV_e	$-\frac{1}{\pi}$	$-\pi$	Ψ_p	$\Delta\Psi_p$	RSWC	ΔRSWC	Elastic modulus ε
bar	bar^{-1}	mg	µl	%	µl	bar^{-1}	--------bar----------			------%-------		bar
0	∞	1239.0*	0	100.00	0	0.1011	9.89	9.89	-	100.00	-	-
2.0	0.5000	1228.7	10.3*	98.93	10.3	0.0999	10.01	8.01	1.88	98.83	1.17	160.68
3.10	0.2439	1221.1	7.6	98.13	17.9	0.0991	10.09	5.99	2.02	97.97	0.86	234.88
6.15	0.1626	1211.5	17.2	96.34	35.1	0.0971	10.30	4.15	1.84	96.02	1.95	94.36
8.20	0.1220	1196.3	15.2	94.75	50.3	0.0953	10.49	2.29	1.86	94.29	1.73	107.51
10.10	0.0990	1171.4	24.9	92.16	75.2	0.0925	10.81	0.71	1.58	91.47	2.82	56.03
11.60	0.0862	1114.6	56.8	86.23	132.0	0.0860	11.63	0.03	0.68	85.02	6.45	10.54
12.80	0.0784	1046.2	68.4	79.09	200.4	0.0781	12.80	0.00	0.03	77.26	7.76	0.39
14.20	0.0704	977.4	68.8	71.92	269.2	0.0702	14.24	0.00	-	69.45	-	-
17.00	0.0588	886.2	91.2	62.40	360.4	0.0598	16.73	0.00	-	59.11	-	-
19.20	0.0521	820.5	65.7	55.55	426.1	0.0522	19.15	0.00	-	51.65	-	-
20.80	0.0481	779.0	41.5	51.22	467.6	0.0475	21.07	0.00	-	46.94	-	-

*Extrapolation from Figure 8.

Dry weight = 288.0 mg

Residual water = 491.0 mg

Bound water = 8.07%

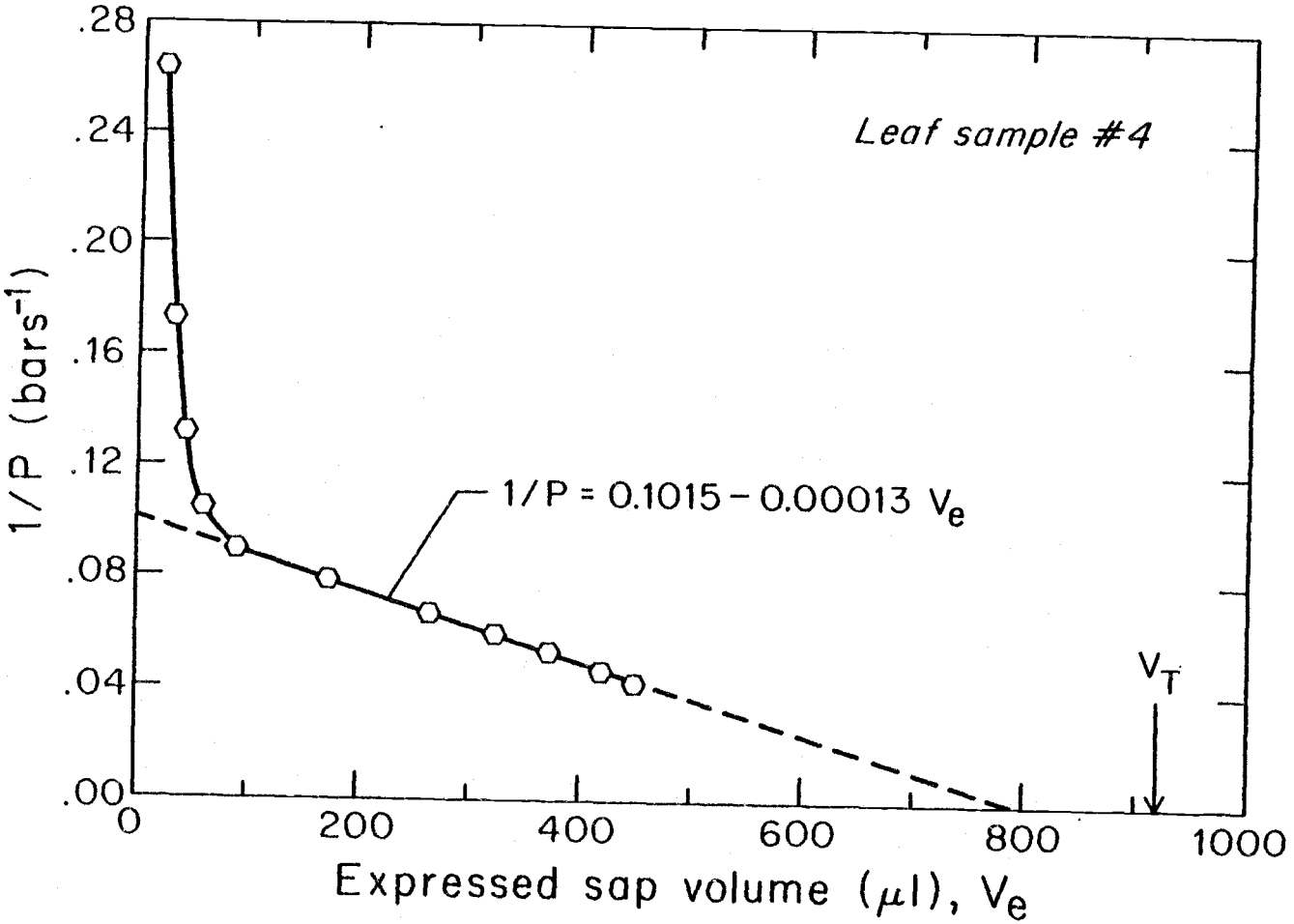

Figure 6. Water release curve of fully expanded first trifoliate leaf (Sample No. 4).

Table 4. Data obtained for construction of pressure-volume relationship of soybean leaf No. 4.

Balanced pressure (P)	$\frac{1}{P}$	Leaf weight	Sap expressed V_e	RWC	ΣV_e	$-\frac{1}{\pi}$	$-\pi$	Ψ_p	$\Delta\Psi_p$	RSWC	ΔRSWC	Elastic *modulus* ε
bar	bar^{-1}	mg	µl	%	µl	bar^{-1}	---------%----------			------%------		bar
0	∞	1193.0*	0	100.00	-	0.1015	9.86	9.86	-	100.00	-	-
1.8	0.5556	1181.5	11.5*	98.74	11.5	0.0995	10.05	8.25	1.61	98.55	1.45	111.03
3.8	0.2632	1171.7	9.8	97.67	21.3	0.0983	10.18	6.38	1.87	97.31	1.24	150.81
5.8	0.1724	1160.8	10.9	96.48	32.2	0.0969	10.32	4.52	1.86	95.93	1.38	134.78
7.6	0.1316	1149.6	11.2	95.26	43.4	0.0954	10.48	2.88	1.64	94.51	1.42	115.49
9.6	0.1042	1133.5	16.1	93.50	59.5	0.9034	10.71	1.11	1.77	92.48	2.03	87.19
10.8	0.0926	1101.3	32.2	89.98	91.7	0.0892	11.21	0.41	0.70	88.41	4.07	17.20
12.8	0.0781	1019.0	82.3	80.98	174.0	0.0787	12.71	0.00	0.41	78.00	10.41	3.94
15.1	0.0662	927.8	91.2	71.01	265.2	0.0915	10.93	0.00	-	66.50	-	-
16.8	0.0600	867.7	60.1	64.44	325.3	0.0593	16.88	0.00	-	58.87	-	-
18.6	0.0538	821.2	46.5	59.36	371.8	0.0533	18.77	0.00	-	53.00	-	-
21.2	0.0472	772.0	49.2	53.98	421.0	0.0470	21.29	0.00	-	46.78	-	-
22.5	0.0424	742.0	30.0	50.71	451.0	0.0431	23.19	0.00	-	42.98	-	-

*Extrapolation from Figure 8.

Dry weight = 278.1 mg

Residual water = 463.9 mg

Bound water = 13.90%

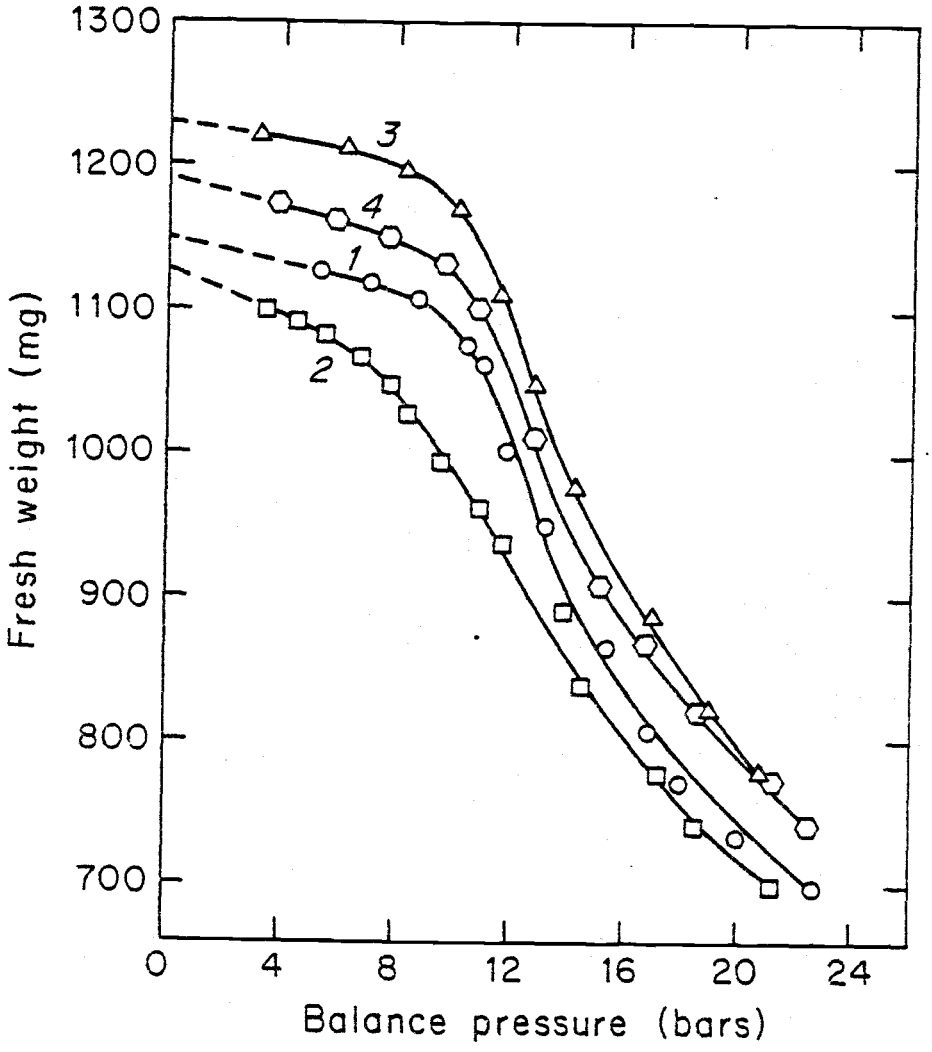

Figure 7. Relationship between fresh weights of fully expanded first trifoliate leaves and corresponding balance pressures (Sample Nos. 1, 2, 3, and 4).

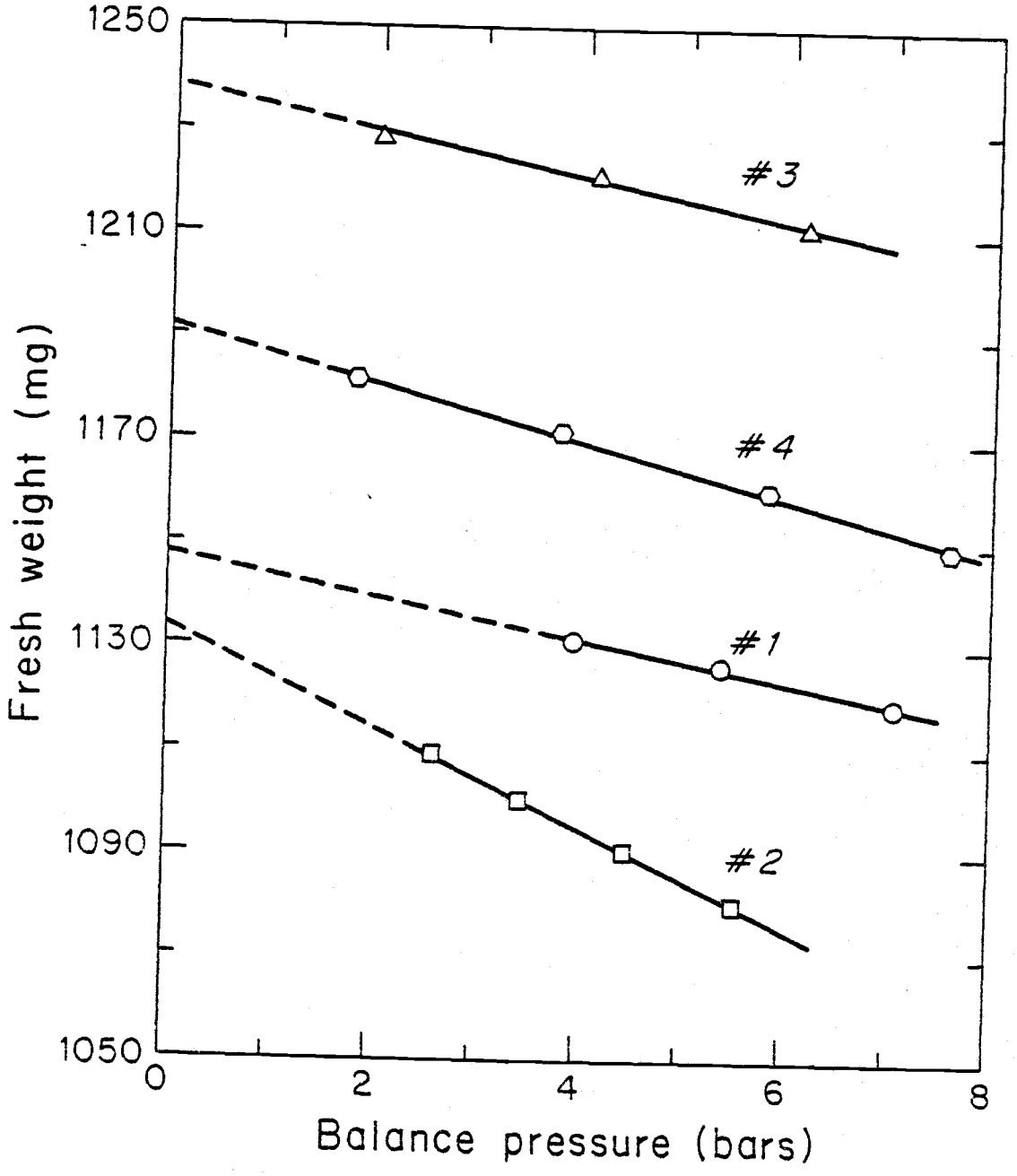

Figure 8. Extrapolations of the relationships between the balance pressures and leaf fresh weights for estimating fresh weight at full turgor (Sample Nos. 1, 2, 3, and 4).

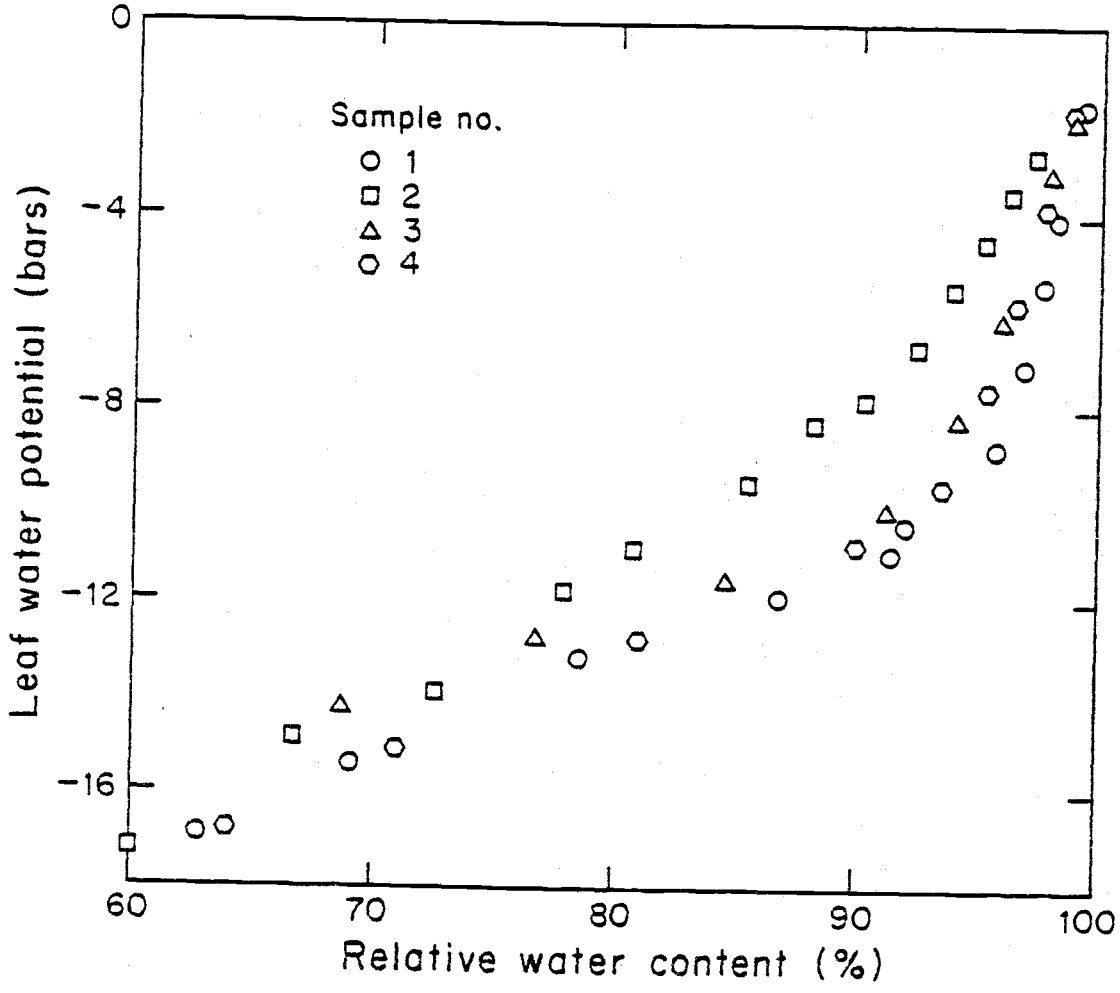

Figure 9. Relationship between plant water potential and relative water content of fully expanded first trifoliate leaves as determined by the P-V method.

Apoplastic Water

The percentage of apoplastic water in the leaf tissue ranged from 7.2 to 13.9 percent of the total volume of water in the tissue with the average value of 9.8 $\pm$ 3.0 percent. This value is within the range found in the literature for non-woody species (Wilson et al., 1980; Campbell et al., 1979). However, Wenkert et al. (1978b) reported a mean apoplastic water content of 16 to 28 percent for immature and mature field grown soybean leaves. The values reported by Wenkert et al. may have been overestimated since they used the water volume at the time of sampling as the total water content instead of the volume of water at full turgor. Wilson et al. (1980) compared the water relations and tissue characteristics of unstressed fully expanded leaves of green panic (Panicum maximum) grown under different environmental conditions. They observed that mean apoplastic water content of plants grown in the controlled environment was 11 percent compared to 13 percent for plants grown in the field under well watered conditions. Cellulose, hemicellulose and lignin content in the cell wall of the plants grown in both environments were similar, although the size of some cells in the field grown leaves were smaller than those of plants grown in the controlled environment.

Rehydration of Leaf Tissues

It was observed from the P-V plots that it was difficult to obtain reproducible data in the range near full turgor. This problem arises because full turgor (zero leaf water potential) is never obtained at the initial pressure chamber reading. Although the cut

had been kept under water and leaves were maintained in a 100% relative humidity environment for 12 hours, the brief period during preparation and transfer of the leaves into the pressure chamber allows evaporation which causes the observed initial leaf water potential to be between 1.7 to 2.7 bars (Tables 1 through 4).

Alternative means of rehydrating the leaf sample were explored. The cut petiole was immersed in distilled water in the pressure chamber with the leaf exposed to the ambient air outside the chamber. The system was pressurized slowly to 2.5 bars until guttation appeared on the leaf surface. This took about half an hour. The chamber was then depressurized and the leaf sample was blotted dry with cotton wool and re-inserted with the leaf inside the chamber for P-V measurements. A similar procedure was used to rehydrate wheat leaves in recent experiments by Campbell et al. (1979).

The initial balance readings using this technique were between 0.15 to 0.20 bar (Tables 5 and 6). Analysis of the P-V data showed that there was a large difference between the amount of water held in the range of 0 to 2.0 bars as estimated by extrapolation of the first few balance pressure readings (Fig. 8) and that obtained by rehydrating the leaves in the pressure chamber (Fig. 11). The amounts of water held in this potential range were 12 $\pm$ 5 mg and from 220 to 270 mg according to the first and second techniques respectively. If we assume that evaporation during the one minute preparation time for the pressure chamber measurements caused a drop in leaf water potential from presumably full turgor to -2.0 bars, the water content of 220 to 270 mg is equivalent to an evaporation rate of 264 to 324 mg/hr/cm^2 considering a leaf area of the fully expanded first

Table 5. Data obtained for construction of pressure-volume relationship of soybean leaf sample No. 5.

Balanced pressure (P)	$\frac{1}{P}$	Leaf weight	Sap expressed V_e	RWC	ΣV_e	$-\frac{1}{\pi}$	$-\pi$	Ψ_p	$\Delta\Psi_p$	RSWC	ΔRSWC	Elastic modulus ε
bar	bar^{-1}	mg	µl	%	µl	bar^{-1}	---------bar---------			------%------		bar
0	∞	1530.6	0	100.00	0	0.1315	7.60	7.60	-	100.00	-	-
0.15	6.6667	1456.0	74.6	94.10	74.6	0.1234	8.10	7.95	-0.35	93.72	6.28	-
0.25	4.0000	1424.3	31.7	91.60	106.3	0.1200	8.33	8.08	-0.13	91.03	2.69	-
0.30	3.3333	1371.8	52.5	87.45	158.8	0.1144	8.74	8.44	-0.36	86.60	4.43	-
0.45	2.2222	1321.9	49.9	83.50	208.7	0.1090	9.18	8.73	-0.29	82.40	4.20	-
0.75	1.3333	1282.1	39.8	80.36	248.5	0.1047	9.55	8.80	-0.07	79.02	3.38	-
1.60	0.6250	1264.0	18.1	78.93	266.6	0.1027	9.73	8.13	0.67	77.49	1.53	43.79
3.20	0.3125	1252.4	11.6	78.01	278.2	0.1015	9.85	6.65	1.48	76.51	0.98	151.02
4.80	0.2083	1236.6	15.8	76.76	294.0	0.0998	10.02	5.22	1.43	75.18	1.33	107.52
8.20	0.1220	1202.2	34.4	74.04	328.4	0.0961	10.41	2.21	3.01	72.27	2.91	103.44
11.40	0.0877	1102.9	99.3	66.19	427.7	0.0853	11.72	0.32	1.89	63.89	8.38	22.55
13.40	0.0746	1002.0	100.9	58.22	528.6	0.0745	13.43	0.03	0.29	55.36	8.53	3.40
15.10	0.0662	928.4	73.6	52.40	602.2	0.0665	15.04	0.00	0.03	49.15	6.21	-
17.50	0.0571	839.8	88.6	45.40	690.8	0.0570	17.56	-	-	41.66	7.49	-
18.60	0.0538	811.6	28.2	43.17	719.0	0.0539	18.55	-	-	39.28	2.38	-

Dry weight = 265.5 mg

Residual water = 546.1 mg

Bound water = 3.69%

Note: Conditions of full turgidity were obtained by placing the cut petiole in the container filled with water inside the pressure chamber.

Table 6. Data obtained for construction of pressure-volume relationship of soybean leaf No. 6.

Balanced pressure (P)	$\frac{1}{P}$	Leaf weight	Sap expressed V_e	RWC	ΣV_e	$-\frac{1}{\pi}$	$-\pi$	Ψ_p	$\Delta\Psi_p$	RSWC	ΔRWCS	Elastic modulus ε
bar	bar^{-1}	mg	µl	%	µl	bar^{-1}	---------bar----------			------%-------		bar
0	∞	1576.7	0	100.00	0	0.1400	7.14	7.14	-	100.00	-	-
0.20	5.0000	1491.4	85.3	93.56	85.3	0.1303	7.68	7.48	-0.34	93.09	6.91	-
0.70	1.4286	1399.6	91.8	86.62	177.1	0.1199	8.34	7.64	-0.16	85.64	7.45	-
3.00	0.3333	1345.8	53.8	82.56	230.9	0.1139	8.79	5.79	1.85	81.28	4.36	42.43
5.60	0.1786	1324.4	21.4	80.95	252.3	0.1113	8.98	3.38	2.41	79.55	1.73	139.31
9.60	0.1042	1240.0	84.4	74.57	336.7	0.1017	9.83	0.23	3.15	72.71	6.84	46.05
11.20	0.0893	1140.0	100.0	67.02	436.7	0.0904	11.06	0.00	0.23	64.60	4.56	5.04
12.60	0.0794	1083.7	56.3	62.77	493.0	0.0840	11.91	-	-	60.04	4.60	-
13.00	0.0769	1027.9	55.8	58.52	549.3	0.0776	12.87	-	-	55.48	4.56	-
14.00	0.0714	985.2	42.7	55.29	592.0	0.0728	13.75	-	-	52.01	3.47	-
14.60	0.0685	938.4	46.8	51.76	638.8	0.0674	14.83	-	-	48.22	3.79	-
15.60	0.0641	896.7	41.7	48.61	680.5	0.0627	15.95	-	-	44.84	3.38	-
17.40	0.0575	851.4	45.3	45.20	725.6	0.0576	17.37	-	-	41.19	3.65	-
18.60	0.0538	827.2	24.2	43.37	749.8	0.0548	18.24	-	-	39.22	1.97	-

Dry weight = 252.9

Residual water = 574.3

Bound water = 6.83%

Note: Conditions of full turgidity were obtained by placing the cut petiole in a container filled with water inside the pressure chamber.

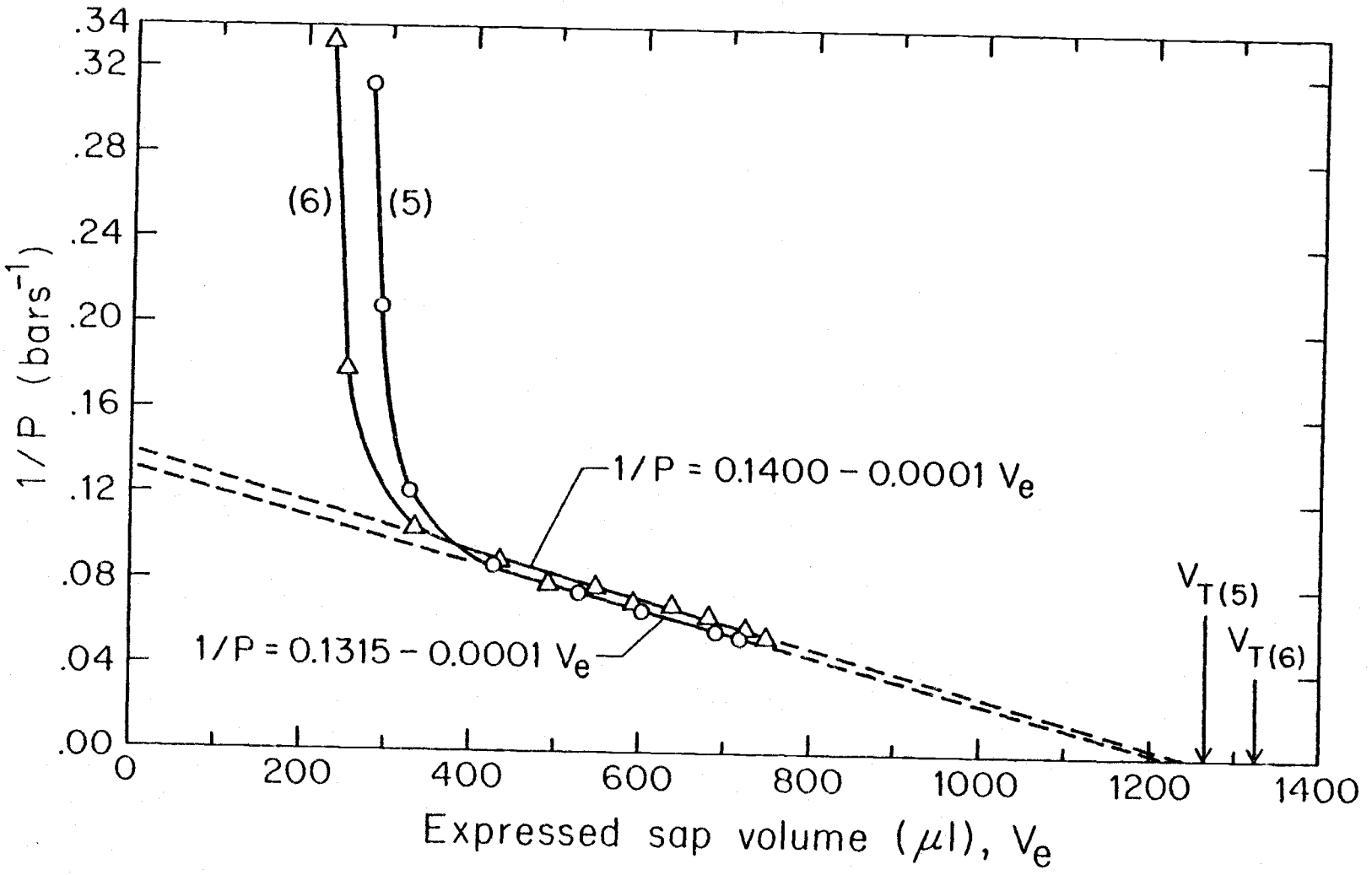

Figure 10. Water release curve of fully expanded first trifoliate leaves (Sample Nos. 5 and 6).

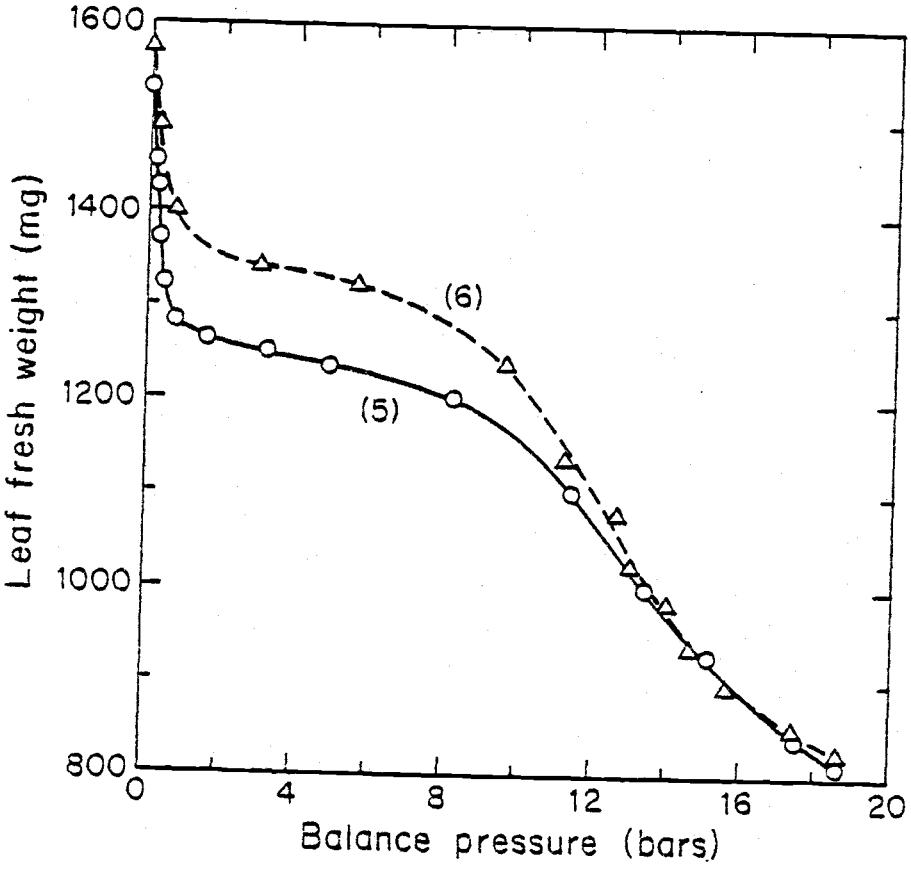

Figure 11. Relationship between fresh weight of fully expanded first trifoliate leaves Nos. 5 and 6 and corresponding balance pressure.

trifoliate leaf of 50.0 cm^2. On the other hand, leaves which were rehydrated by submerging the cut petioles overnight under humidified and dark conditions retained an estimated amount of water of 12 $\pm$ 5 mg between full turgor and -2.0 bars, according to the extrapolation method shown in Figure 8. The loss of water by this amount in one minute is equivalent to a transpiration rate of 14.4 $\pm$ 6.0 mg/hr/cm^2. This figure is very close to the transpiration rate of 18.0 mg/hr/cm^2 from soybean leaves under well watered conditions in a controlled environment chamber (Boyer, 1970b) and 22.4 mg/hr/cm^2 reported by Kuo and Boersma (1971). Rate of water loss was low in this study because P-V measurements were conducted in the laboratory where the light intensity was lower than in the growth chamber, although leaf water potentials under consideration were higher than in the experiments of the above researchers.

It is clear that rehydration of leaves in the pressure chamber as used with other plant species (Campbell, 1979) is not suitable for soybean leaves. This is because excess water probably accumulates in the air spaces next to mesophyll cells as a result of pressurization. In contrast, the estimates of fresh weight at full turgor obtained by the extrapolation method (Ladiges, 1975) seemed to agree well with transpiration rates from soybean leaves reported in the literature. This technique was therefore used to determine fresh weights at full turgor which in turn were used in subsequent calculations of the parameters reported in Tables 1 through 4.

The consequences of rehydrating leaves inside the pressure chamber were very dramatic. The P-V curves of the leaves rehydrated by this method (Fig. 10) shifted away from the ordinate, π_o and π_p were less negative, and zero turgor potential occurred at lower

relative water contents than obtained when using leaves which were rehydrated overnight. Percentages of apoplastic water of leaves subjected to rapid rehydration were also lower, namely 3.7 to 6.8 percent, mainly because excess water added up to a higher total volume of water in the tissues.

The most serious errors brought about by excess water which occurred with pressurizing technique are demonstrated by the π and Ψ_p data estimated from the P-V curves (Tables 5 and 6). There was an increase in Ψ_p when leaf water potential (balance pressure) decreased from 0 to -0.75 bar followed by a decrease until zero turgor potential was reached. This resulted in negative values of $\Delta\Psi_p$ and therefore led to negative values of the elastic modulus. The negative elastic modulus and the increase in Ψ_p with decreasing leaf water content cannot physically occur in the plant. The problem is caused by the high estimated value of π_o (less negative) as a result of the excess water in the leaf at full turgor.

Elastic Modulus

Equation (11) was used to calculate the elastic modulus of leaf tissue for each paired measurement. The results are shown in Tables 1 through 4. There was an apparent drop in the elastic modulus near full turgor. This is considered to be an artifact caused by uncertainty in the extrapolations involved in obtaining the amount of water retained in this leaf water potential range. A relatively small error in estimating the water content can produce large errors in ε because of the very rapid decrease in turgor potential per unit amount of water expressed near zero potential or full turgor (Cheung et al., 1976).

Relationships between ε and Ψ_p are shown in Figure 12. The general form of the relation between ε on Ψ_p agrees with results reported by Cutler et al. (1979), Cheung et al. (1976), Wenkert et al. (1978b), and Steudle et al. (1977). The maximum value of ε ranged from 76 to 235 bars, with an average of 174 $\pm$ 76 bars. For mature soybean leaves in the field, Wenkert et al. (1978b) found that the mean initial elastic modulus at the time of sampling was 184 $\pm$ 61 bars. They also reported that ε decreased linearly with decreasing turgor potentials. Steudle et al. (1977) used a pressure probe technique to measure ε of M. crystallinum and of giant algae (Valonia utricularis) cells directly. They found that ε increased in linear fashion with Ψ_p in the low turgor pressure range whereas at higher pressure ε approached a constant value. The data can be fitted by a function of the form,

$$\varepsilon = \varepsilon_m \{1 - \exp(-\Psi_p/k)\} \tag{13}$$

Where ε_m is the maximum value for the elastic modulus, and k is the turgor potential at which $\varepsilon/\varepsilon_m = 1 - 1/e$. The linear form of Equation (13) is

$$\ln(\varepsilon_m - \varepsilon) = \ln\varepsilon_m - \Psi_p/k \tag{14}$$

Landsberg (1977) described a procedure to fit experimental data with a function similar to Equation (13). If the value of ε_m was not clearly indicated from the measurement data, both ε_m and k can be estimated graphically. The technique involves guessing the value of ε_m and plotting $\ln(\varepsilon_m - \varepsilon)$ as a function of Ψ_p. If the selected value of ε_m is too large the plot is nonlinear and concaves towards the Ψ_p axis

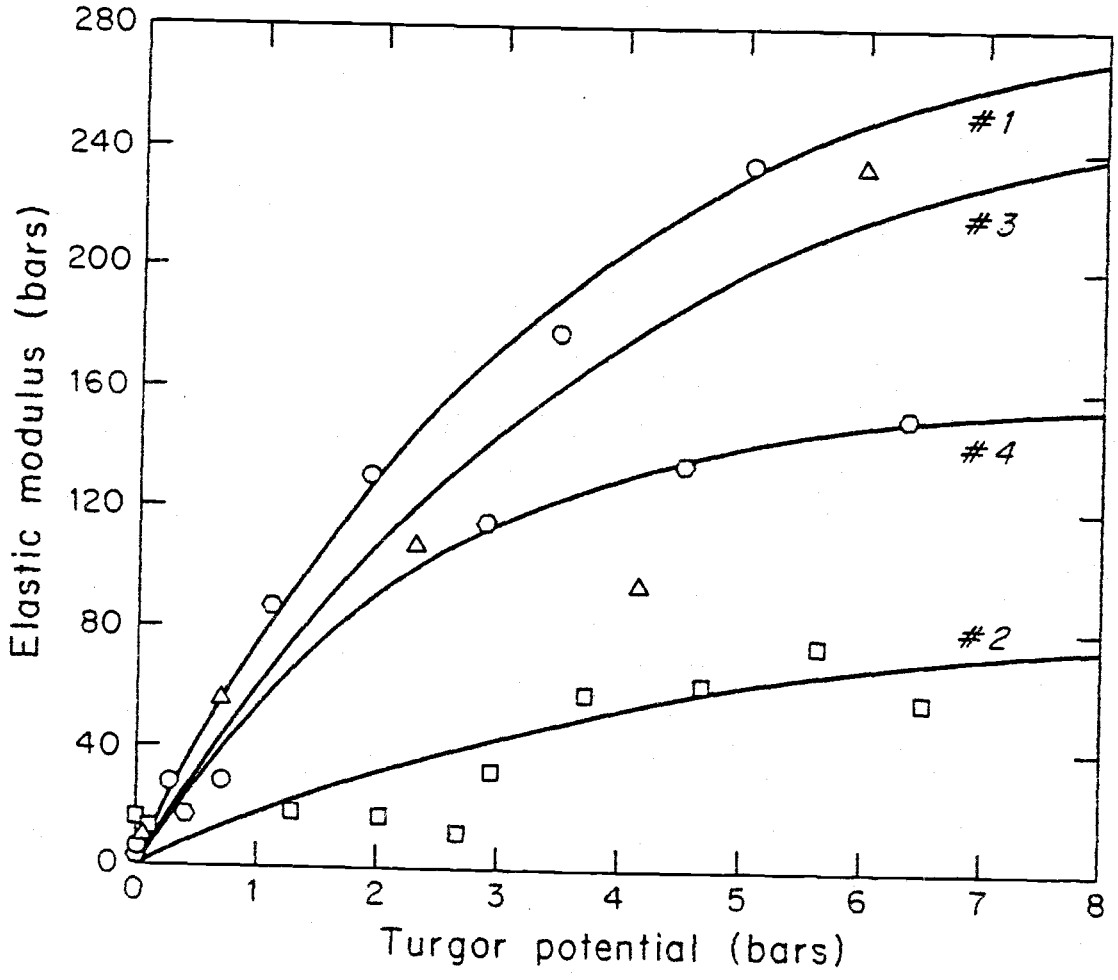

Figure 12. Relationship between elastic modulus and turgor potential of soybean leaf samples. Open symbols represent measured data, the solid lines are drawn from the calculated data using Equation (13) and values of ε_m and 1/k from Figures 13 and 14.

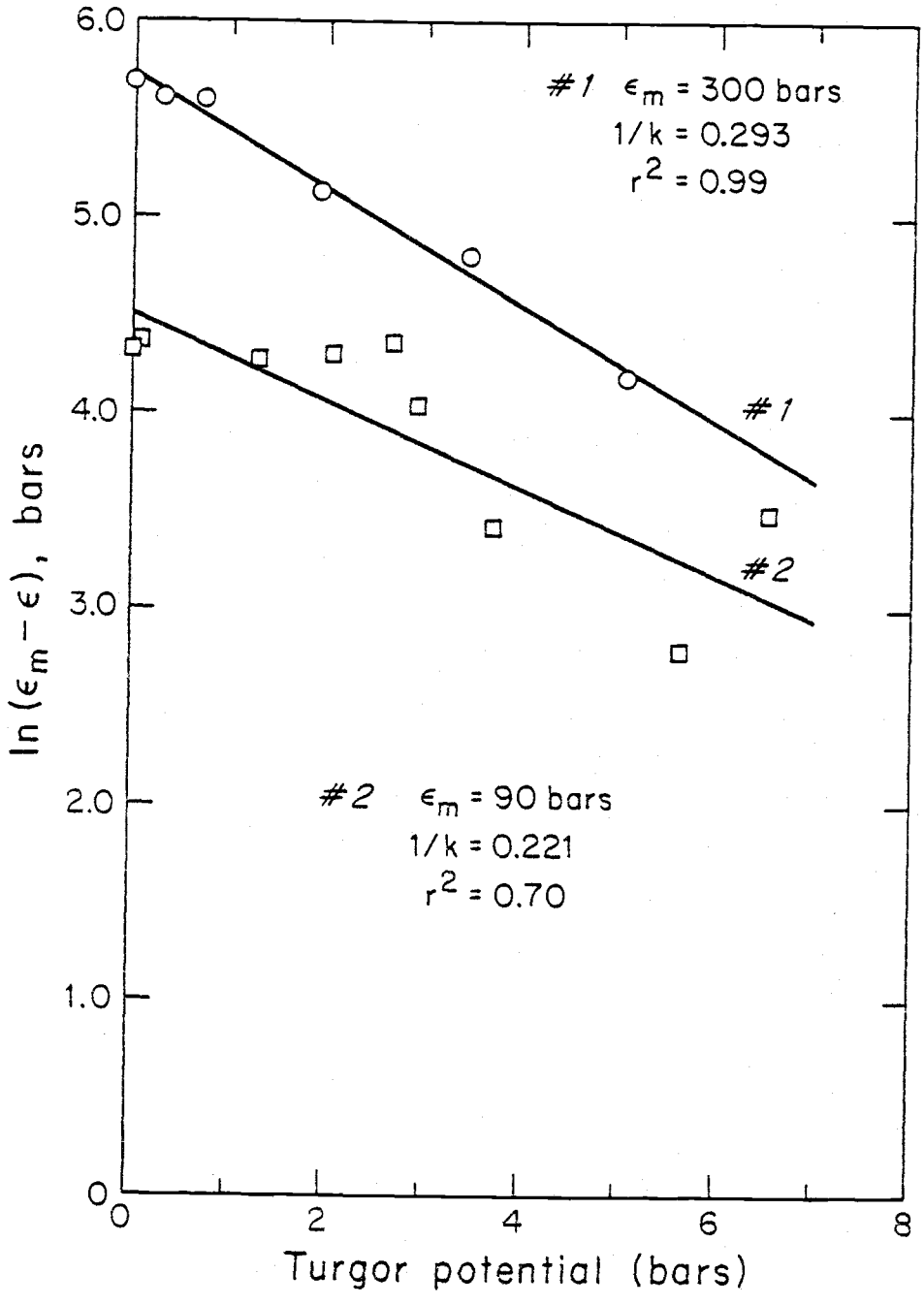

Figure 13. Plots of $\ln(\varepsilon_m - \varepsilon)$ versus turgor potential using the values of ε_m obtained by trial and error as described in the text, for soybean leaf Sample Nos. 1 and 2.

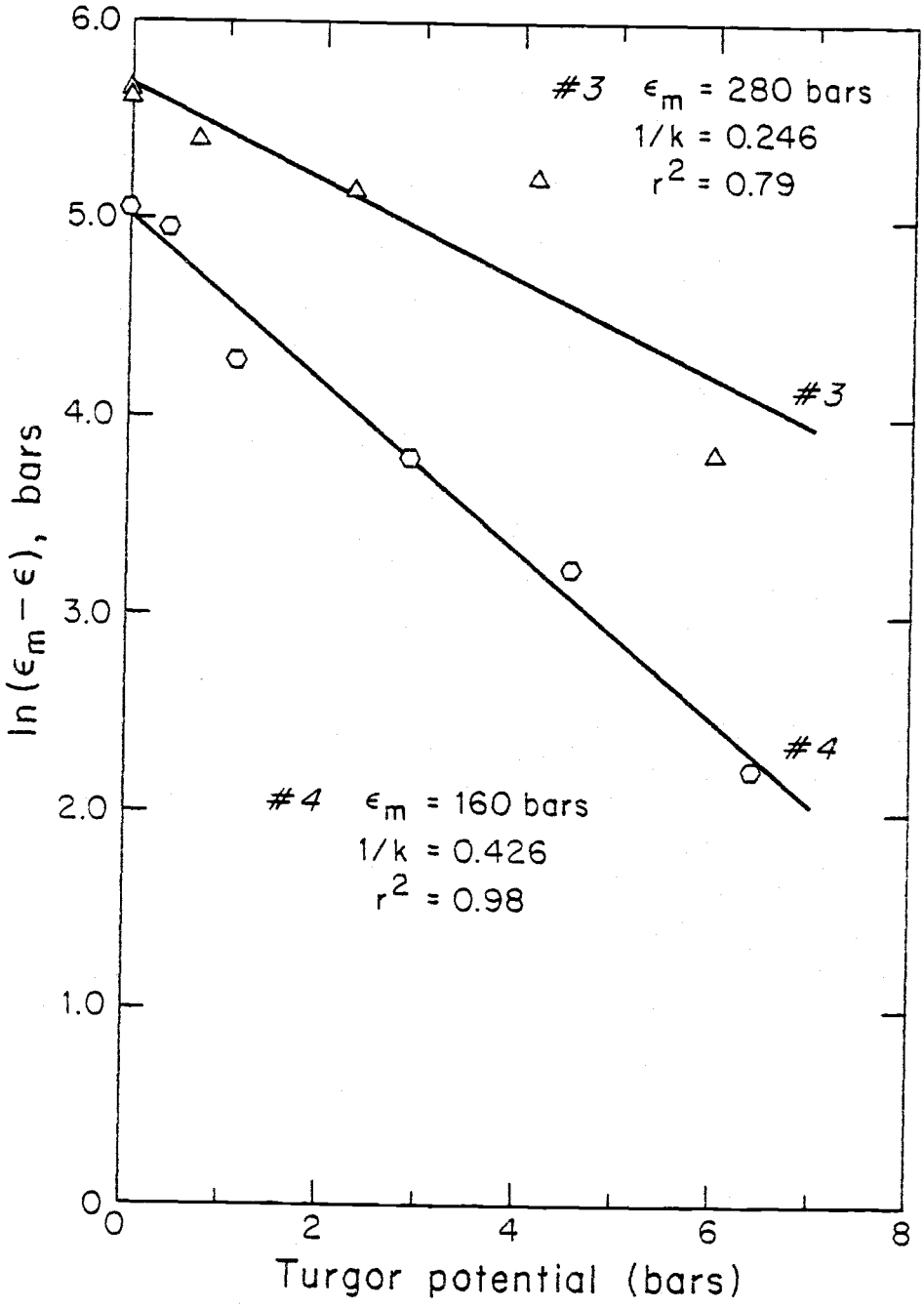

Figure 14. Plots of $\ln(\varepsilon_m - \varepsilon)$ versus turgor potential using the values of ε_m obtained by trial and error as described in the text, for soybean leaf Sample Nos. 3 and 4.

and if it is too small the curve concaves upward. Choices of ε_m are made until a linear plot is obtained and the intercept with the $\ln(\varepsilon_m - \varepsilon)$ axis is at the value close to $\ln\varepsilon_m$. The best value of ε_m is thus obtained. The slope of this plot is 1/k according to Equation (14).

The procedure described by Landsberg was used for plotting $\ln(\varepsilon_m - \varepsilon)$ vs Ψ_p using data shown in Tables 1 through 4. Data points near full turgor which were based on extrapolations to obtain volume of water were not included. The plots of $\ln(\varepsilon_m - \varepsilon)$ vs Ψ_p constructed with ε_m obtained by trial and error as described above are shown in Figures 13 and 14. The best values of ε_m obtained by the graphical method ranged from 90 to 300 bars with an average 207.5 $\pm$ 99.8 bars, and k ranged from 2.35 to 4.52 bars with an average 3.59 $\pm$ 0.94 bars.

The smaller the value of k, the better the ability of the plant cells to maintain the same turgor potential over a range of water potentials providing the same ε_m applies. A high ε value has physiological advantages. The relative change in water content of a cell with a high ε value is smaller than in a cell with a low ε value for the same change in turgor potential. Equation (10) illustrates this point.

The estimated values of ε_m and k from Figures 13 and 14 were used in Equation (13) to calculate ε at increment values of Ψ_p. Results of these calculations are plotted in Figure 12. Although the experimental data seemed to fit the function in Equation (13) reasonably well (r^2 ranged from 0.70 to 0.99), variations in the values of ε_m and k for individual leaves grown under the same environmental condition may limit the use of Equation (13) for

predicting ε from known turgor potentials. A similar range of variation in initial elastic modulus of soybean leaves was reported by Wenkert et al. (1978b). Steudle et al. (1977) demonstrated that ε does not only depend on Ψ_p but also on cell volume. The higher values of ε_m were measured on cells with the greater cell volume at full turgidity, both in giant algae and in higher plant species.

The reasons for variation in the relationship between ε and Ψ_p of soybean leaves remains unclear. However, it is noticed that the higher values of ε of leaf samples 1 and 3 corresponded to volume at full turgidity (853 and 881 µl respectively, Figs. 3 and 5). Samples 2 and 4 with smaller ε values had symplastic volumes at full turgidity of 800 and 791 µl respectively (Figs. 4 and 6). For a better understanding of the relationship between ε and Ψ_p a more detailed study involving a larger number of samples grown at different environmental conditions is suggested.

Effects of Soil Water Potential and Soil Temperature on Plant Water Status

Leaf Water Potential

Lowering the soil water potential resulted in a decrease of soybean leaf water potential at both soil temperatures of 25 and 10°C (Table 7). There was a significant interaction between soil temperature and soil water potential as suggested by the analysis of variance (Appendix Table 2). However, the F-value observed for the soil water potential treatment was many times greater than for the temperature x water potential interaction. Therefore the main effects of soil water potential on leaf water potential can be

Table 7. Leaf water potential, osmotic potential, and turgor potential as affected by soil water potential and soil temperature.

Soil temperature	Soil water potential	Leaf water potential	Osmotic potential		Turgor potential	
			Uncorrected	Corrected	Uncorrected	Corrected
°C	bars					
25	-0.35	- 6.4	-12.2	-13.7	5.8	7.3
25	-2.5	- 9.2	-12.9	-14.4	3.7	5.3
25	-5.0	-12.6	-15.1	-17.1	2.5	4.5
10	-0.35	- 8.2	-12.4	-14.0	4.2	5.8
10	-2.5	-12.9	-15.2	-17.3	2.3	4.4
10	-5.0	-14.4	-16.1	-18.4	1.6	4.0
	$LSD_{.05}$*	0.7	0.5	0.7	0.7	1.0
	$LSD_{.01}$*	1.0	0.7	0.9	1.0	1.4

*LSD to compare soil water potential means for the same soil temperature.

Table 8. Main effect of soil water potential on leaf water potential, osmotic potential, and turgor potential of the first trifoliate leaves of 18-day old soybean seedlings.

Soil water potential	Leaf water potential	Osmotic potential		Turgor potential	
		Uncorrected	Corrected	Uncorrected	Corrected
		bars			
-0.35	- 7.3	-12.3	-13.8	5.0	6.5
-2.5	-11.1	-14.1	-15.9	3.0	4.8
-5.0	-13.5	-15.6	-17.8	2.1	4.3
$LSD_{.05}$	0.5	0.4	0.5	0.5	0.7
$LSD_{.01}$	0.7	0.5	0.7	0.7	1.0

Table 9. Main effect of soil temperature on leaf water potential, osmotic potential, and turgor potential of the first trifoliate leaves of 18-day old soybean seedlings.

Soil temperature	Leaf water potential	Osmotic potential		Turgor potential	
		Uncorrected	Corrected	Uncorrected	Corrected
°C	bars				
25	- 9.4	-13.4	-15.0	4.0	5.7
10	-11.8	-14.6	-16.6	2.7	4.7

summarized as in Table 8. The decrease in leaf water potential from -7.3 to -13.5 bars with the decrease in soil water potential from -0.35 to -5.0 bars suggest that under the constant evaporative demand of the growth chamber the semipermeable membrane and osmotic solution functioned satisfactorily in controlling water potential in the soil and plant system.

Table 9 shows that the leaf water potential was lowered by 2.4 bars when the soil temperature was decreased from 25 to 10°C. This decrease in leaf water potential was not caused by a decrease in the water potential of the PEG solution due to the low temperature of the osmotic solution. Temperature effects on potential of the osmoticum were accounted for in the preparation of the solution using Equation (2). It is likely that the increase in the viscosity of the plant sap and the corresponding decrease in water uptake were responsible for the observed decrease in leaf water potential at low soil temperature (Dalton and Gardner, 1978; Ungs et al., 1982).

Osmotic Potentials

Both uncorrected and corrected values of osmotic potential are shown in Table 7. Uncorrected values are reported so that data can be compared with results in the older literature on plant water relations. Main effects of soil water potential (Table 8) show that there was a significant decrease in osmotic potential from -12.3 to -14.1 and to -15.6 bars when the soil water potential decreased from -0.35 to -2.5 and to -5.0 bars respectively. A similar main effect of decreasing soil water potential on corrected osmotic potential occurred. The results suggest that solute concentrations in the leaf

tissue increased either by reduced volume of cell water, or by accumulation of more osmotically active solutes.

Turgor Potential

A significant drop in turgor potentials occurred when the soil water potential decreased from -0.35 to -5.0 bars (Table 8). The magnitude of the decrease in turgor potential was less than that of the leaf water potential. This indicates that osmotic adjustment occurred in the plants as a result of accumulation of solutes at lower leaf water potentials.

Uncorrected and corrected turgor potentials (Table 7) and turgor potentials obtained from P-V measurements (Tables 1 through 4) were plotted against corresponding leaf water potentials (Fig. 15). Linear relationships between Ψ_p and Ψ were found with r^2 values of 0.98, 0.96 and 0.99 for uncorrected and corrected turgor potential, and Ψ_p estimated from P-V measurements respectively. The slope of the lines in Figure 15, $d\Psi_p/d\Psi$, indicates the degrees of osmotic adjustment of the leaves. A value approaching 1.0 indicates that osmotic adjustment does not occur. A decrease in the value of $d\Psi_p/d\Psi$ indicates the degree of turgor pressure maintenance. The slope of the relationship obtained by the P-V method (Fig. 15) is 0.83. It implies that no osmotic adjustment occurred during the rapid removal of water from the leaf samples in the pressure chamber. The theory of P-V measurement includes the assumption that a decrease in osmotic potential due to removal of water from the tissues, results from the removal of water and not from the accumulation of solutes (Tyree, 1972). The fully expanded first trifoliate leaves

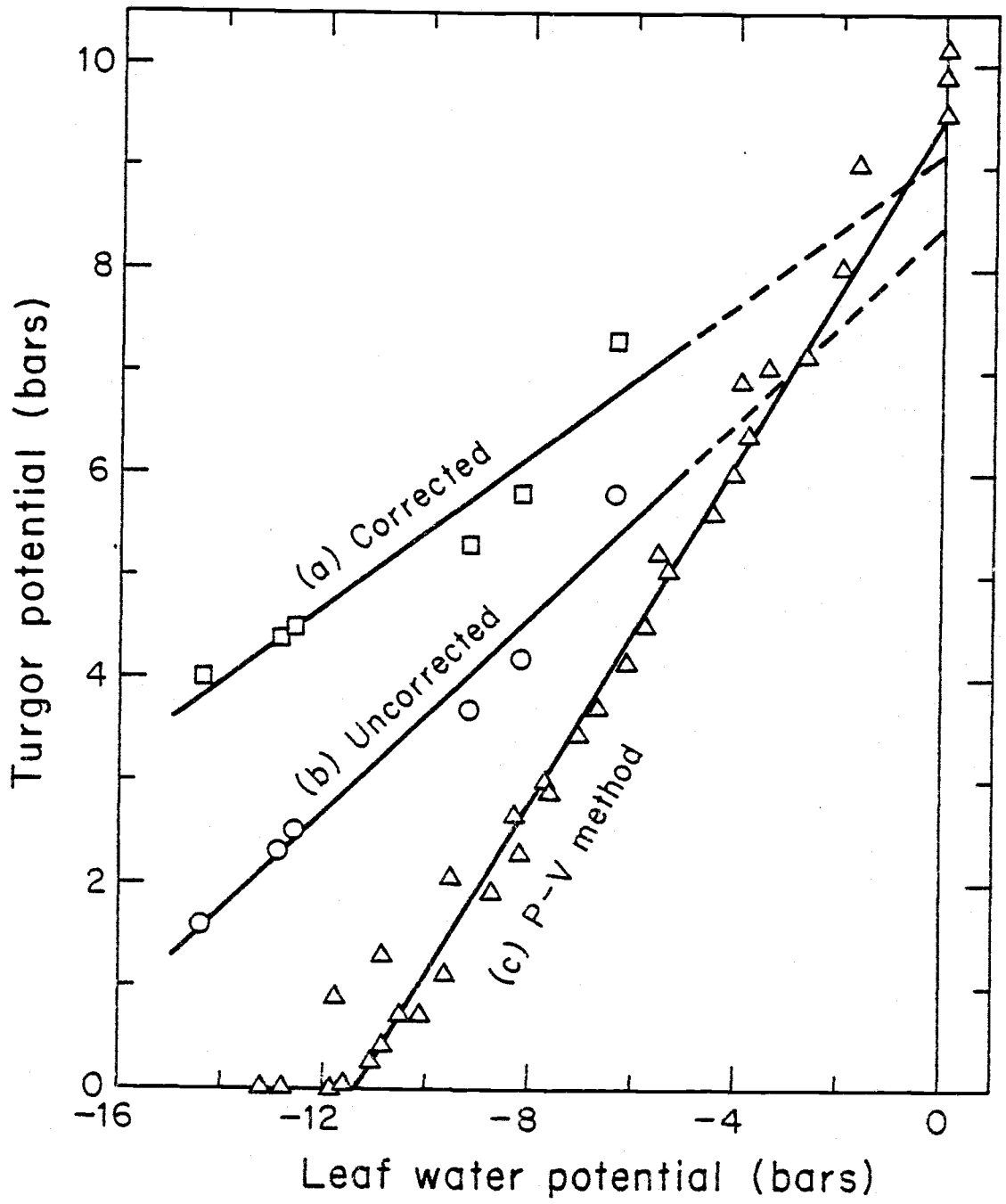

Figure 15. Relationships between leaf water potential and turgor potential for fully expanded first trifoliate leaves obtained by different methods of estimating turgor potentials.

exhibited a certain degree of osmotic adjustment. The slopes for the plots were 0.48 for the uncorrected turgor potential and 0.37 for the corrected turgor pressure data. Turner and Jones (1980) reviewed the evidences for partial turgor maintenance by osmotic adjustment. They found that $d\Psi_p/d\Psi$ varied from 0.29 to 0.57 for well watered plants which were subsequently allowed to dry slowly and depended on plant species and cultivar.

Useful information emerges from Figure 15 that deserves further discussion. With a decrease in leaf water potential, turgor potential estimated by the P-V technique appeared to be smaller than the turgor potential obtained as the difference between leaf water potential measured by the pressure chamber and the osmotic potential measured by the dew point hygrometer. This is indicated by the steeper slope of line (c) compared to lines (a) and (b) in Figure 15. The reason is that Ψ_p values used in plotting lines (a) and (b) were obtained from the plants growing in a natural environment outside the pressure chamber. In this condition the plants have time to accumulate solutes for the osmotic adjustment to occur. The rapid loss of water from the leaves in the pressure chamber does not permit plants to accumulate the osmotically active solutes in the vacuoles. The determination of Ψ and Ψ_p from P-V measurements is therefore questionable, particularly on plants which are known to have osmotic adjustment capability.

An osmotic adjustment index (OI) is proposed in the present report. It is defined as the difference between the slope of the relationship between turgor potential and leaf water potential obtained

by the P-V technique and that determined on the plants which had been allowed to dry slowly, divided by the former quantity.

$$OI = \left(\frac{d\Psi_p^{\,v}}{d\Psi^v} - \frac{d\Psi_p}{d\Psi}\right) / \left(d\Psi_p^{\,v}/d\Psi^v\right) \tag{15}$$

or

$$OI = 1 - \frac{d\Psi_p/d\Psi}{d\Psi_p^{\,v}/d\Psi^v} \tag{16}$$

where $d\Psi p^v/d\Psi^v$ and $d\Psi_p/d\Psi$ are the slopes of relationship between turgor potential and leaf water potential obtained by drying plants growing in soil in a natural environment, and that obtained from P-V measurement. The range of the OI is from 0 to 1.0. The value of 1.0 indicates full turgor maintenance by means of osmotic adjustment while the zero value means that no osmotic adjustment occurs.

Turner and Jones (1980) used the difference in the slope of the relationship between turgor potential and water potential ($d\Psi_p/d\Psi$) between rapidly dried, well-watered plants and plants allowed to dry slowly as an index of osmotic adjustment. The rapid drying was achieved by severing the stems at the soil surface. The values of $d\Psi_p/d\Psi$ for rapidly drying leaves, as reviewed in their paper, varied from 0.50 to 0.88 depending on plant species and growing conditions. In comparing the relative effects of experimental treatments on degree of osmotic adjustment within and among plant species, the osmotic adjustment index defined by Equation (16) seems to be more appropriate than the difference in value of $d\Psi_p/d\Psi$ between slowly and rapidly drying plants as used by Turner and Jones. The reason is that the value of the OI is normalized using the reference slope, $d\Psi_p^{\,v}/d\Psi^v$, obtained from the P-V curve, the theory of which is based on the assumption of

no osmotic adjustment in the leaf. Moreover, the turgor potential obtained by the P-V technique as used here can be obtained without further measurement of osmotic potential of tissue sap by the hygrometric method which suffers from errors due to dilution of solutes by apoplastic water caused by disruption of cell walls.

Extrapolation of the corrected Ψ_p to zero leaf water potential (Fig. 15, line b) showed that Ψ_p at full turgor agreed within 0.5 bar with the value determined by the P-V method.

The effect of lowering the soil temperature on Ψ_p was similar to the response to decreasing soil water potential. Turgor potential decreased by 1.0 bar whereas leaf water potential decreased by 2.4 bars when the soil temperature was lowered from 25 to 10°C. The partial turgor maintenance was achieved by a decrease in osmotic potential (Table 9).

Effects on Plant Growth

Leaf Elongation Rate

The rates of growth of the second, third, and fourth trifoliate leaves were severely affected by soil water potential. The elongation rates of the second trifoliate leaves were reduced from 11.0 to 1.0 mm/day when soil water potential decreased from -0.35 to -5.0 bars (Table 10). The third and fourth trifoliate leaves which were parts of the tips of the soybean seedlings behaved similarly. Their rates of elongation decreased from 13.3 and 1.6 mm/day respectively at the soil water potential of -0.35 bar to apparently no growth at -5.0 bars. The reduction in growth of growing tissue due to water stress is well documented (Boyer, 1970; Hsiao, 1973; Barlow et al., 1977). Bunce

Table 10. Rate of leaf elongation of 18-day old soybean seedlings as a function of soil water potential and soil temperature.

Soil temperature	Soil water potential	Rate of leaf elongation 2nd leaves	3rd leaves	4th leaves
^{o}C	bars	----------mm/day----------		
25	-0.35	11.0	13.3	1.6
25	-2.5	1.0	1.0	0.0
25	-5.0	1.9	0.5	0.0
10	-0.35	1.3	1.9	0.3
10	-2.5	0.0	0.3	0.0
10	-5.0	0.0	0.0	0.0
	$LSD_{.05}$*	0.9	1.8	0.4
	$LSD_{.01}$*	1.3	2.5	0.7

*LSD to compare soil water potential means for the same soil temperature.

(1977) showed a linear relationship between rates of elongation of soybean leaves and turgor pressure in three different environments. Elongation rates of the expanding leaves during the day decreased from 0.8 to 0.1 mm/hr when turgor potential decreased from 5.2 to 2.0 bars. A similar trend was observed in the present study. Elongation rates of the third trifoliate leaves decreased sharply from 0.55 to 0.02 mm/hr when the uncorrected leaf turgor potential decreased from 5.8 to 2.5 bars at a soil temperature of 25°C and under continuous light (Tables 7 and 10).

Effects of soil temperature on elongation rates of the expanding leaves are shown in Table 10. At the soil water potential of -0.35 bar, elongation rates of the second, third and fourth trifoliate leaves were 1.3, 1.9 and 0.3 mm/day respectively with a soil temperature of 10°C. With a soil temperature of 25°C the corresponding rates were 11.0, 13.3, and 1.6 mm/day. The expanding leaves ceased to elongate at 10°C at both soil water potential treatments of -2.5 and -5.0 bars. The decrease in leaf water potential can partly explain the reduced leaf elongation rate at low root temperature. The cessation of leaf growth at the leaf water potentials below -12.9 bars is in agreement with results reported by Boyer (1970) which showed that leaf enlargement of soybeans stopped when leaf water potential was about -14.0 bars.

Effects of Soil Water Potential and Soil Temperature on Partitioning of ^{14}C

Total ^{14}C Activities

Total activities of ^{14}C expressed as dpm present in whole plants grown at the different treatment conditions are shown in Table 11. Values shown are the sums of absolute activities in each plant part. Activities in mature primary leaves were not included in the calculation of total activities because only trace amounts had been detected during earlier experimental runs. These tissues were not analyzed in later experiments.

Analysis of variance for these data indicates that the effects of sampling time after labeling on total ^{14}C activities was not significant (Appendix Table 10). The main effects of soil water potential are summarized in Table 12. No significant interactions between Temperature x Potential x Time and between Temperature x Potential were observed (Appendix Table 10). Fixation of $^{14}CO_2$ was reduced by 20 and 33 percent when soil water potential decreased from -0.35 to -2.5 and -5.0 bars respectively. Main effects of soil temperature suggest that plants grown at the soil temperature of $10^{o}C$ fixed 30 percent less $^{14}CO_2$ than plants grown at the root temperature of $25^{o}C$ (Table 13).

Total ^{14}C activities were also expressed as dpm per mg of tissue dry weight to account for variations in dry weights of the plant parts used in the experiments (Tables 14 and 15). Results of this analysis showed similar effects of soil water potential.

The effects of decreasing leaf water potential on net photosynthesis have been demonstrated in the literature. Rate of

Table 11. Total ^{14}C activities (dpm) in 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm x 10^{-3}					
25	-0.35	1602	1740	1866	1808	1760	1741
25	-2.5	1427	1363	1318	1427	1559	1491
25	-5.0	1282	1213	1241	1286	1332	1333
10	-0.35	1377	1342	1337	1333	1301	1302
10	-2.5	1175	938	986	1008	1022	963
10	-5.0	716	798	828	746	811	865

$LSD_{.05*}$ = 210

$LSD_{.01*}$ = 282

*LSD to compare soil water potential means for the same soil temperature and the same or different times after labeling.

Table 12. Main effects of soil water potential on total ^{14}C activities (dpm) in 18-day old soybean seedlings.

Soil water potential	^{14}C activities
bar	dpm x 10^{-3}
-0.35	1543
-2.5	1223
-5.0	1038
$LSD_{.05}$	56
$LSD_{.01}$	82

Table 13. Main effect of soil temperature on total activities (dpm) in 18-day old soybean seedlings.

Soil temperature	^{14}C activities
^{o}C	dpm x 10^{-3}
25	1488
10	1047

Table 14. Total ^{14}C activities in 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm/mg					
25	-0.35	8736	9664	9396	9409	10031	9082
25	-2.5	8278	8282	7779	8101	8083	8531
25	-5.0	8656	8518	7660	8334	8618	7818
10	-0.35	7314	7401	6982	7508	6773	7152
10	-2.5	7082	6139	6003	6819	6422	6269
10	-5.0	4993	5763	5375	5047	5418	5547

$LSD_{.05*} = 1062$

$LSD_{.01*} = 1456$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Table 15. Main effect of soil water potential on total ^{14}C activities (dpm/mg) in 18-day old soybean seedlings.

Soil water potential	Total ^{14}C activities
bar	dpm/mg
-0.35	8287
-2.5	7316
-5.0	6812
$LSD_{.05}$	450
$LSD_{.01}$	654

Table 16. Main effect of soil temperature on total ^{14}C activities (dpm/mg) in 18-day old soybean seedlings.

Soil temperature	Total ^{14}C activities
^{o}C	dpm/mg
25	8610
10	6334

photosynthesis in soybeans was not affected by leaf water potential as low as -11.0 bars (Boyer, 1970). Rate of photosynthesis of corn continuously decreased with a decrease in leaf water potential. In these experiments the total ^{14}C activities in the plants growing at $10^{o}C$ were 74 percent of those growing at $25^{o}C$ (Table 16).

Labeled First Trifoliate Leaves

Recently fixed ^{14}C continued to move from the fully expanded first trifoliate leaves, even 24 hours after labeling. The activities of ^{14}C remaining in the source leaves expressed as dpm/mg are shown in Table 17. Analysis of variance of these data (Appendix Table 12) indicates that there was no significant difference between soil water potential treatments. However, the data when presented in this manner do not truly reflect the relative rate of export of photosynthates from the labeled leaves in response to experimental treatments. Activities of ^{14}C have more commonly been expressed in translocation studies either as percentage of total dpm or percentage of total dpm/mg of plant part. Expression of ^{14}C translocation from the labeled leaves as percentage of total amount absorbed seems to be most appropriate in this study because the total ^{14}C activities were affected by both soil water potential and soil temperature. Similar effects of treatments on ^{14}C activities remaining in the labeled leaves were observed whether activities were expressed as percentage of total dpm or percentage of total dpm/mg (Tables 18 and 19).

Analysis of variance for ^{14}C activities (% of total dpm/mg) remaining in the labeled first trifoliate leaves (Appendix Table 14)

Table 17. Activities of ^{14}C (dpm/mg) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm/mg					
25	-0.35	7481	7946	7296	6155	5689	3846
25	-2.5	7664	6816	6039	5796	5333	4399
25	-5.0	8542	7997	6776	7199	7054	4510
10	-0.35	7011	6698	5662	5631	4874	4334
10	-2.5	6924	5789	5112	5392	4987	4042
10	-5.0	4954	5588	4858	4240	4224	3941

Table 18. ^{14}C activities (% of total dpm) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	----------% of total dpm----------					
25	-0.35	85.84	78.33	77.03	67.83	56.73	39.15
25	-2.5	91.87	82.54	76.32	72.27	67.34	54.21
25	-5.0	98.05	91.61	85.66	84.63	79.73	53.94
10	-0.35	95.18	89.53	83.73	76.79	75.26	63.97
10	-2.5	96.84	92.18	84.97	82.61	79.50	67.26
10	-5.0	98.94	95.66	90.01	84.35	81.00	71.76

$LSD_{.05*} = 6.49$

$LSD_{.01*} = 8.73$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

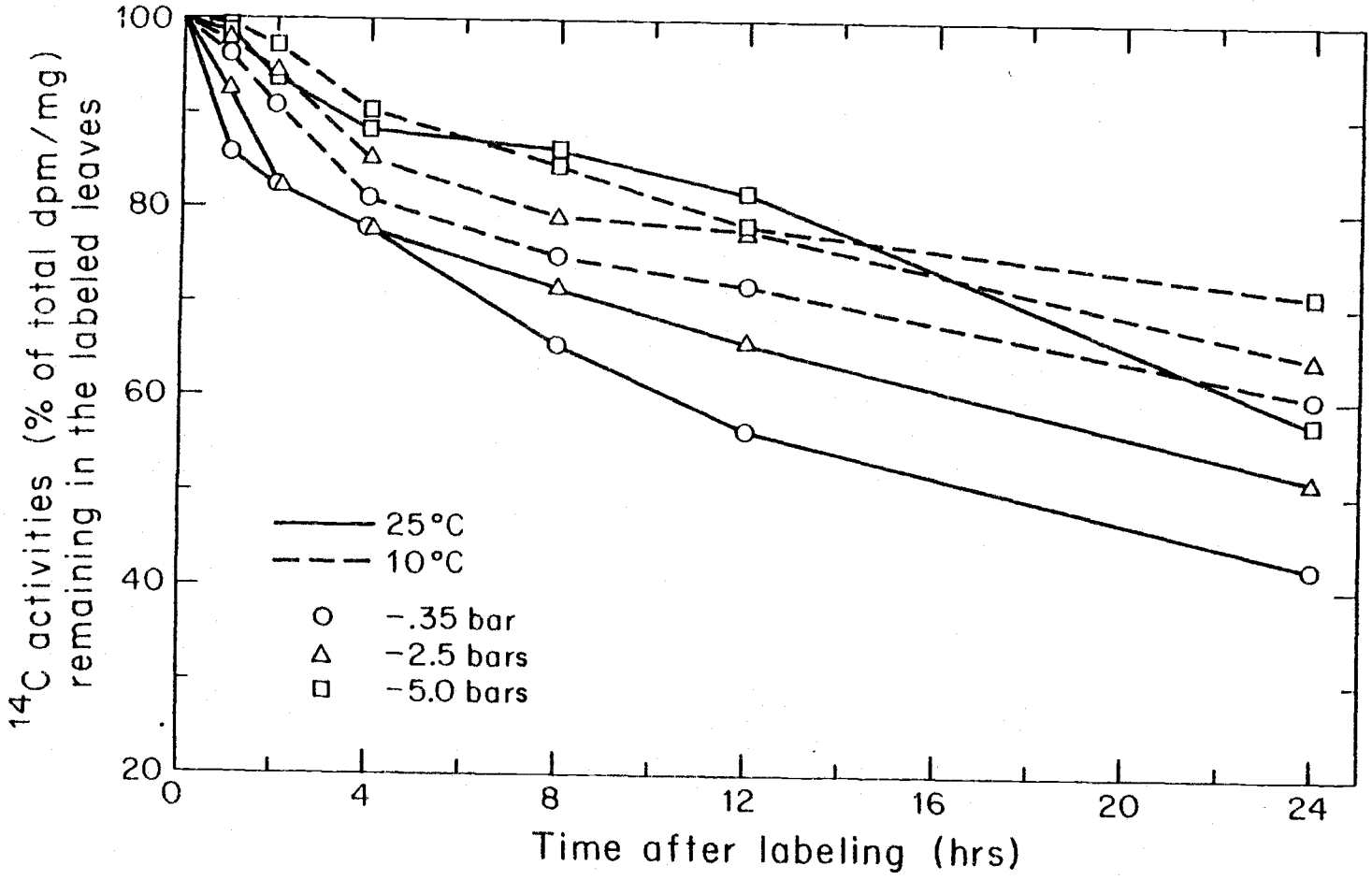

Figure 16. Activities of ^{14}C (percentage of total dpm/mg) remaining in the labeled first trifoliate leaves as a function of soil water potential, soil temperature and time after labeling.

Table 19. Activities of ^{14}C (% of total dpm/mg) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	----------% of total dpm/mg----------					
25	-0.35	85.76	82.30	77.63	65.43	56.36	42.21
25	-2.5	92.54	82.26	77.68	71.53	65.90	51.54
25	-5.0	98.54	93.85	88.05	86.25	81.79	57.65
10	-0.35	96.14	90.74	81.01	74.92	71.92	60.21
10	-2.5	97.75	94.12	85.22	79.03	77.65	64.45
10	-5.0	99.23	96.98	90.16	84.48	77.99	71.18

$LSD_{.05*} = 5.97$

$LSD_{.01*} = 8.01$

*LSD to compare water potential means for the same soil temperature and same or different times after labeling.

indicates that there was an interaction between soil temperature and soil water potential.

At soil temperatures of 25°C the rates of decrease in ^{14}C activities in the labeled first trifoliate leaves were greatest during the first 2 hours after labeling followed by a period of slower rate of decrease throughout the rest of the 24 hour period. The labeled first trifoliate leaves retained a higher percentage of ^{14}C at all hours after labeling when the soil water potential was decreased from -0.35 to -5.0 bars. At the end of 2 hours the labeled leaves of the plants growing at -0.35 bar retained 82 percent of total dpm/mg compared to 94 percent of total dpm/mg in the leaves at -2.5 bars. At 8 and 24 hours after labeling, ^{14}C activities remaining in the labeled leaves at the -0.35 bar treatment were 65 and 42 percent of total dpm/mg respectively as compared to 86 and 58 percent total dpm/mg in the labeled leaves of the plants growing at -5.0 bars at the corresponding times after labeling.

When the soil temperature was kept at 10°C, the period of rapid decrease in ^{14}C remaining in the labeled leaves was delayed to 4 hours after labeling. There was no significant difference between ^{14}C activities remaining in the labeled leaves of the plants growing at -0.35 and -2.50 bars (Table 19). However, when the soil water potential was decreased to -5.0 bars, labeled leaves retained more ^{14}C at all times after labeling than the leaves at -0.35 bar. At the end of 4 hours labeled leaves of plants growing at the soil water potential of -0.35 bar retained 81 percent of total dpm/mg compared to 90 percent of total dpm/mg at the -5.0 bars treatment. At the end of 24 hours 60 percent of total dpm/mg remained in the labeled

leaves at -0.35 bar and 71 percent of total dpm/mg remained in the labeled leaves at the -5.0 bars treatment.

Second Trifoliate Leaves

Only small percentages of the absorbed ^{14}C were exported to the second trifoliate leaves. Main effects of soil water potential (Table 21) showed only significant differences between the -0.35 and -5.0 treatments at 8 and 12 hours after labeling. There were no differences among the treatment effects at 24 hours. Effects of soil water potential did not show up in the partitioning to the second trifoliate leaves. Those leaves had reached about two-thirds of their fully expanded sizes at the time of the experiment. At this stage of growth soybean leaves change from importing to exporting photosynthates (Thaine et al., 1959). In comparison to the other parts of the plants very low percentages of ^{14}C were in these leaves (Table 20).

A decrease in ^{14}C imported by the second trifoliate leaves due to the main effects of soil temperature was observed (Table 22). At 24 hours after labeling, ^{14}C activities were 1.5 percent of total dpm/mg at 25°C and 0.3 percent at 10°C. At 10°C, elongation of the second trifoliate leaves had stopped (Table 10). The low sink activities at 10°C suggested by the elongation rate data can explain the low activities of ^{14}C found in the second trifoliate leaves if the imported ^{14}C were to partially supply the demand for photosynthates of the growing tissue in these leaves.

Table 20. Activities of ^{14}C (% of total dpm/mg) in the second trifoliate leaves of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	----------% of total dpm/mg----------					
25	-0.35	0.11	0.11	0.39	1.12	1.46	1.61
25	-2.5	0.07	0.05	0.23	0.52	0.87	1.71
25	-5.0	0.02	0.02	0.04	0.18	0.22	1.09
10	-0.35	0.05	0.06	0.07	0.19	0.26	0.46
10	-2.5	0.03	0.03	0.03	1.09	0.12	0.19
10	-5.0	0.05	0.15	0.26	0.05	0.10	0.18

$LSD_{.05*} = 0.61$

*LSD to compare water potential means for the same soil temperature and same or different times after labeling.

Table 21. Main effect of soil water potential on ^{14}C activities (% of total dpm/mg) in the second trifoliate leaves of 18-day old soybean seedlings at different times after labeling.

Soil water potential	Hours after labeling					
	1	2	4	8	12	24
bar	----------% of total dpm/mg----------					
-0.35	0.08	0.09	0.23	0.65	0.86	1.04
-2.5	0.05	0.04	0.13	0.80	0.50	0.95
-5.0	0.03	0.09	0.15	0.11	0.16	0.64

$LSD_{.05*} = 0.43$

*LSD to compare soil water potential means for the same or different times after labeling.

Table 22. Main effect of soil temperature on ^{14}C activities (% of dpm/mg) in the second trifoliate leaves of 18-day old seedlings at different times after labeling.

Soil temperature	Hours after labeling					
	1	2	4	8	12	24
^{o}C	----------% of total dpm/mg----------					
25.0	0.06	0.06	0.22	0.60	0.85	1.47
10.0	0.04	0.08	0.12	0.44	0.16	0.28

Tip

Table 23 shows ^{14}C activities (% of total dpm/mg) in the tips of soybeans at different treatment levels. Analysis of variance in Appendix Table 16 indicates that the interaction between soil water potential and soil temperature treatments on ^{14}C activities in the tip was highly significant.

At the soil temperature of 25°C, plants growing at the soil water potential of -0.35 bar imported 2.7 percent of total dpm/mg in the tip at the end of the first hour after labeling (Table 23 and Fig. 17). The tip continued to accumulate ^{14}C photosynthate at the average rate of about 1 percent of total dpm/mg/hr during the next seven hours, then the rate of ^{14}C accumulation in the tip declined. At the end of the 24 hour period 14.8 percent of total dpm/mg was in the tip at -0.35 bar. Plants growing at the soil water potential of -2.5 bars imported less ^{14}C in the tip at all times after labeling than plants growing at -0.35 bar. The rate of importation of ^{14}C by the tip changed with time after labeling. However the change was not the same at all treatments. The tips of plants growing at the soil water potential of -5.0 bars imported only trace amounts of ^{14}C during the first 2 hours then began to increase slowly until 12 hours after labeling at which time the rate of importation began to increase (Fig. 17). At the end of 24 hours ^{14}C activities in the tip of the plants at -5.0 bars was about 6.0 percent of total dpm/mg.

The pattern of ^{14}C importation by the tips of the plants was somewhat different at the soil temperature of 10°C. The amounts of ^{14}C imported were very low at this temperature. There was no

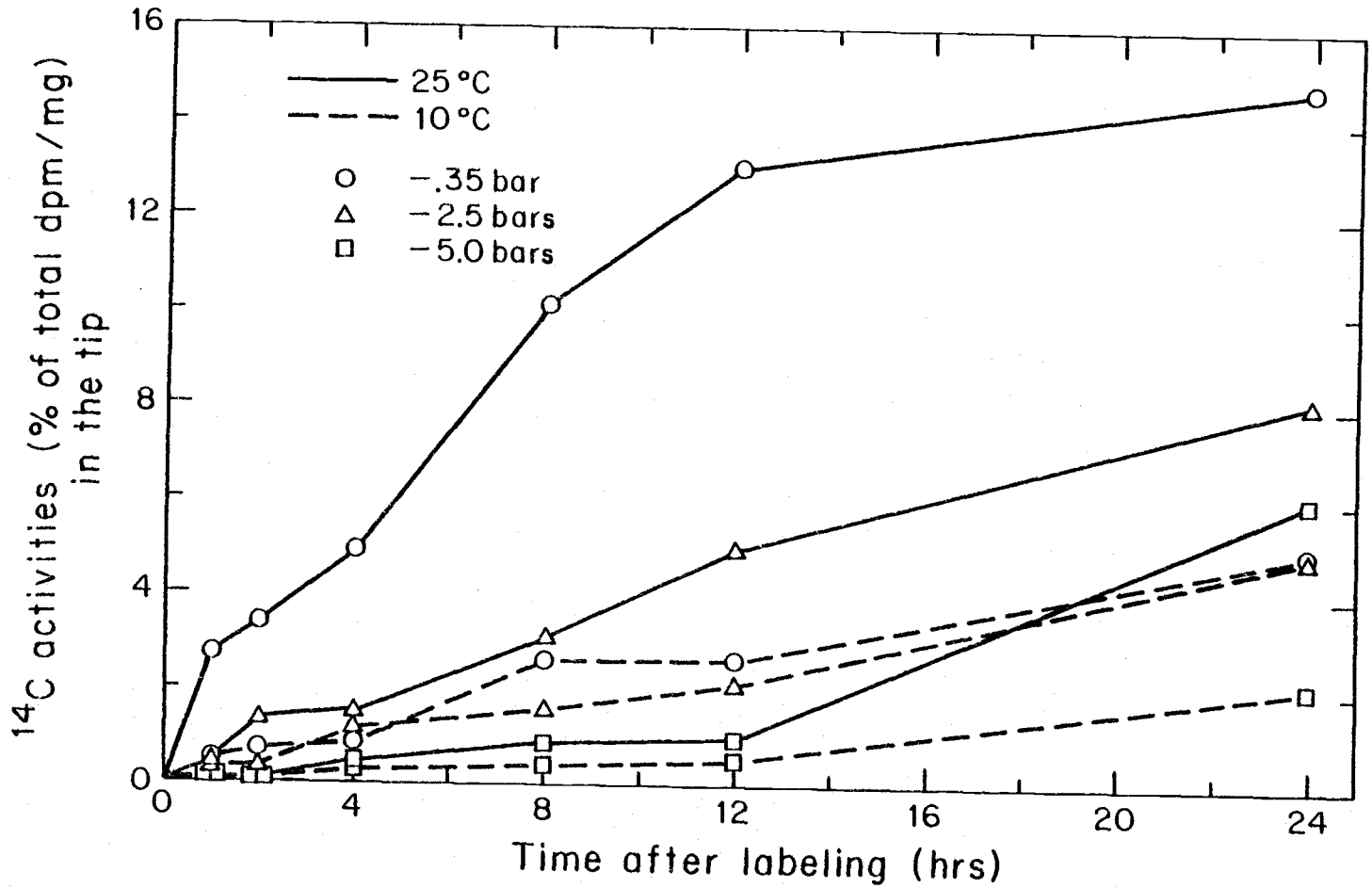

Figure 17. Activities of ^{14}C (percentage of total dpm/mg) in the tip of soybean seedlings as a function of soil water potential, soil temperature and time after labeling.

Table 23. Activities of ^{14}C (% of total dpm/mg) in the tips of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	----------% of total dpm/mg----------					
25	-0.35	2.74	3.44	4.93	10.13	13.03	14.77
25	-2.5	0.41	1.38	1.57	3.11	5.07	8.14
25	-5.0	0.05	0.18	0.43	0.87	0.99	6.03
10	-0.35	0.48	0.79	0.88	2.61	2.65	4.89
10	-2.5	0.12	0.26	1.27	1.54	2.15	4.90
10	-5.0	0.04	0.09	0.29	0.40	0.50	2.11

$LSD_{.05*} = 2.42$

$LSD_{.01*} = 3.29$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

significant difference between ^{14}C activities in the tips of plants at -0.35, -2.5 and -5.0 bars at any times after labeling except at 24 hours. At this sampling time the tips of the plants growing at soil water potentials of -0.35 and -2.5 bars accumulated more ^{14}C than those at the -5.0 bars treatment.

Stem

Activities of ^{14}C (% of total dpm/mg) in the stem are shown in Table 24 and Figure 18. Analysis of variance in Appendix Table 17 indicates that there was a highly significant interaction between soil temperature and soil water potential but the F values of the soil water potential treatments were about 10 times greater than those for the interaction between soil water potential and soil temperature. Thus main effects of soil water potential on ^{14}C activities in the stem can be summarized as in Table 25. The difference between the amount of ^{14}C (% of total dpm/mg) in the stems of plants growing at the soil water potentials of -0.35 and -5.0 bars was highly significant at 1.0 percent level at all hours after labeling. At the end of the first hour 5 percent of total dpm/mg was in the stems of plants growing at the soil water potential of -0.35 bar. This percentage of ^{14}C activities continued to increase but at the slower rate than during the first hour. Activities of ^{14}C in the stem at 8 and 24 hours were 10 and 15 percent of total dpm/mg respectively.

Translocation of ^{14}C from the labled leaves to the stem was delayed when the plants were subjected to a soil water potential of -5.0 bars. Only 0.8 percent of total dpm/mg was in the stems of plants growing at this treatment at the end of the first hour after

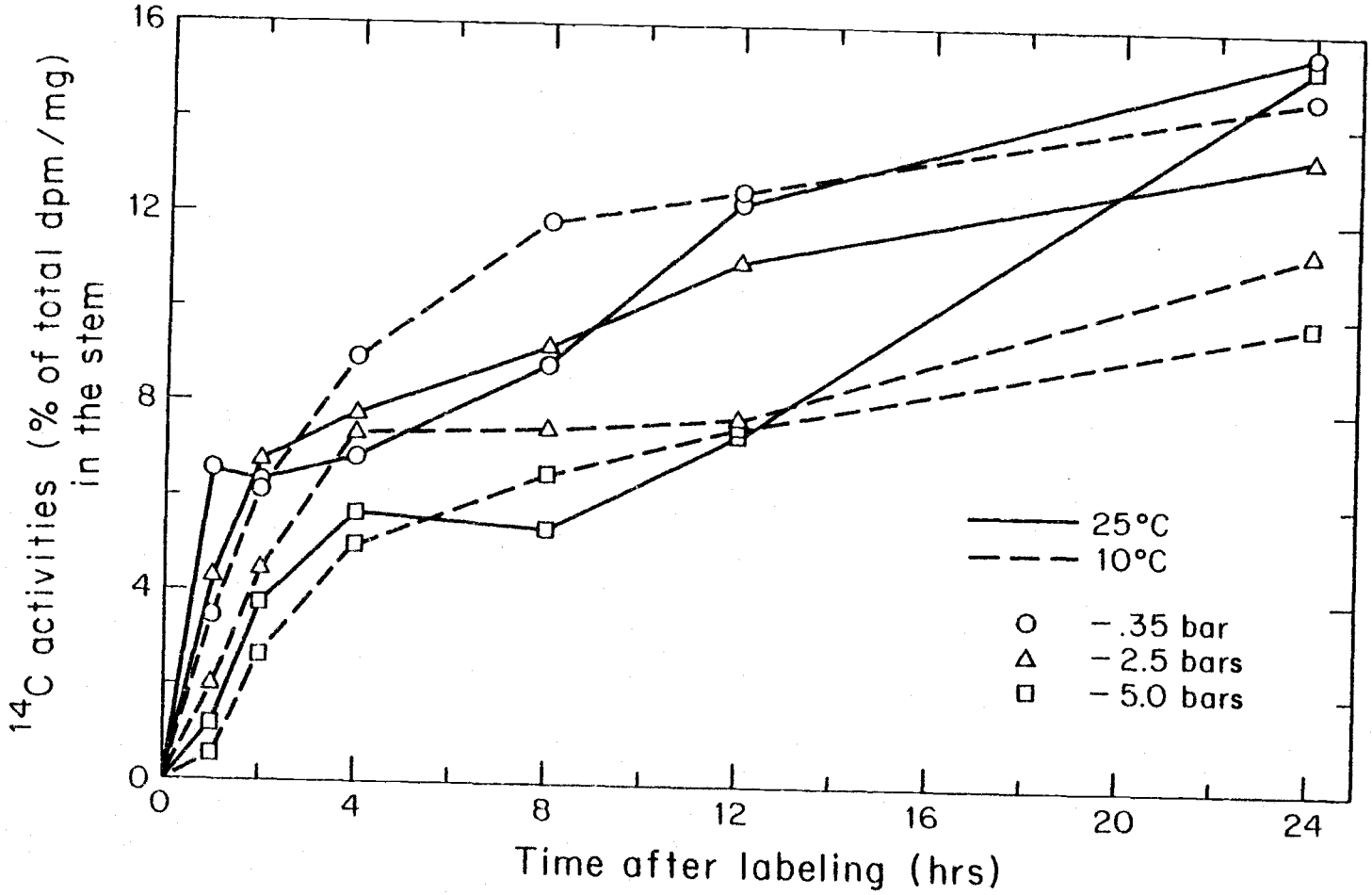

Figure 18. Activities of ^{14}C (percentage of total dpm/mg) in the stem of soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Table 24. Activities of ^{14}C (% of total dpm/mg) in the stems of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
^{o}C	bar	----------% of total dpm/mg----------					
25	-0.35	6.56	6.33	6.86	8.81	12.24	15.51
25	-2.5	4.25	6.72	7.76	9.19	10.99	13.37
25	-5.0	1.14	3.73	5.65	5.34	7.38	15.29
10	-0.35	3.43	6.17	8.95	11.81	12.45	14.61
10	-2.5	2.00	4.42	7.35	7.44	7.62	11.31
10	-5.0	0.50	2.63	4.97	6.50	7.47	9.82

$LSD_{.05*} = 2.36$

$LSD_{.01*} = 3.18$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Table 25. Main effect of soil water potential on ^{14}C activities (% of total dpm/mg) in the stems of 18-day old soybean seedlings at different times after labeling.

Soil water potential	Hours after labeling					
	1	2	4	8	12	24
bar	----------% of total dpm/mg----------					
-0.35	5.00	6.25	7.91	10.31	12.34	15.06
-2.5	3.13	5.57	7.56	8.32	9.30	12.34
-5.0	0.82	3.18	5.31	5.92	7.43	12.56

$LSD_{.05*} = 1.67$

$LSD_{.01*} = 2.25$

*LSD to compare soil water potential means for the same or different times after labeling.

labeling. However, the amount of ^{14}C was 3.2 percent of total dpm/mg at the end of 2 hours and increased to 5.9 and 12.6 percent of total dpm/mg at the end of 8 and 24 hours respectively.

Soil temperature did not appear to affect the amount of ^{14}C in the stem when the same soil water potential levels were compared (Table 24), except at -5.0 bars where ^{14}C activities decreased from 15.3 to 9.8 percent of total dpm/mg 24 hours after labeling when soil temperature decreased from 25 to 10°C.

Roots

Effects of soil water potential, soil temperature, and time after labeling on ^{14}C activities in the roots are shown in Table 26 and Figure 19. Analysis of variance (Appendix Table 18) indicates that there was a highly significant interaction between soil water potential and soil temperature on ^{14}C activities in the roots.

At the soil temperature of 25°C, there was a significant difference between percentage of ^{14}C imported by the roots at -0.35 bar and -5.0 bars at all sampling times except at 24 hours. Time courses of ^{14}C activities in the roots at the three soil water potential treatments and the soil temperature of 25°C were similar to those for the stem (Fig. 18). During the first two hours there were sharp increases in percentage of ^{14}C in the roots. After this period the rates of increase in the percentage of ^{14}C gradually declined. For plants growing at the soil water potential of -5.0 bars, there was a delay in importation of ^{14}C by the roots. At 2 hours after labeling only 1.5 percent total dpm/mg of ^{14}C were in the roots at 5.0 bars compared to 5.2 percent of total dpm/mg in the roots of plants at

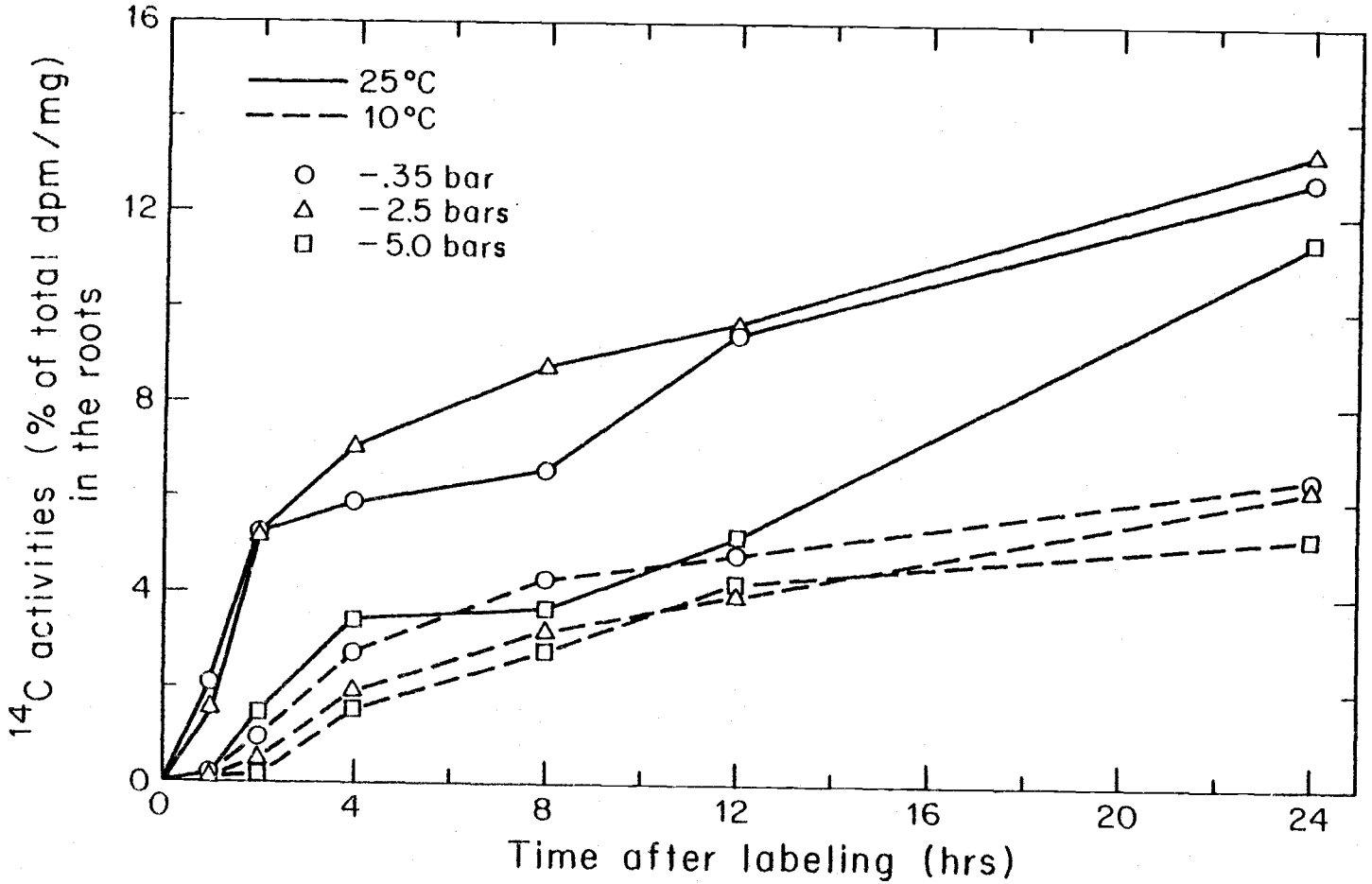

Figure 19. Activities of ^{14}C (percentage of total dpm/mg) in the roots of soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Table 26. Activities of ^{14}C (% of total dpm/mg) in the roots of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	----------% of total dpm/mg----------					
25	-0.35	2.09	5.21	5.88	6.57	9.49	12.72
25	-2.5	1.56	5.18	7.02	8.78	9.61	13.32
25	-5.0	0.13	1.46	3.38	3.67	5.17	11.46
10	-0.35	0.16	0.96	2.72	4.24	4.80	6.47
10	-2.5	0.09	0.49	1.92	3.21	3.96	6.28
10	-5.0	0.12	0.12	1.53	2.79	4.15	5.24

$LSD_{.05*} = 1.80$

$LSD_{.01*} = 2.43$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

soil water potentials of -0.35 and -2.5 bars during the same period (Fig. 19 and Table 26). The rates of increase in percentage of ^{14}C during 4 to 12 hours were similar between different soil water potential treatments. During 12 to 24 hours after labeling the rate of ^{14}C importation at -5.0 bars began to increase. At the end of 24 hours 11.5 percent of total dpm/mg were in the roots of plants at soil water potential of -5.0 bars as compared to 12.7 and 13.3 percent of total dpm/mg at -0.35 and -2.5 bars respectively.

At $10^{o}C$ there was no significant difference between the soil water potential treatments in percentage of ^{14}C imported by the roots. Comparisons between soil temperature effects at the same soil water potential show that ^{14}C activities were consistently lower in the roots of plants at the soil temperature of $10^{o}C$ at all times after labeling.

Nodules

Activities of ^{14}C (% of total dpm/mg) in the nodules are in Table 27. Analysis of variance (Appendix Table 19) indicated that there was no significant interaction between soil water potential and soil temperature. Main effects of soil water potential are in Table 28. There was no significant difference between percentages of ^{14}C in the nodules at -0.35 and -2.50 bars. However, at -5.0 bars the percentage of ^{14}C in the nodules was significantly lower than at -0.35 bar and at -2.50 bars. A delay in the importation of ^{14}C by the nodules at -5.0 bars was demonstrated (Table 28). Only 0.4 percent of total dpm/mg was present in the nodules at -5.0 bars at the end of 2 hours. The corresponding percentages of ^{14}C at -0.35 and and -2.5 bars were 1.9 and 2.6 percent of total dpm, respectively.

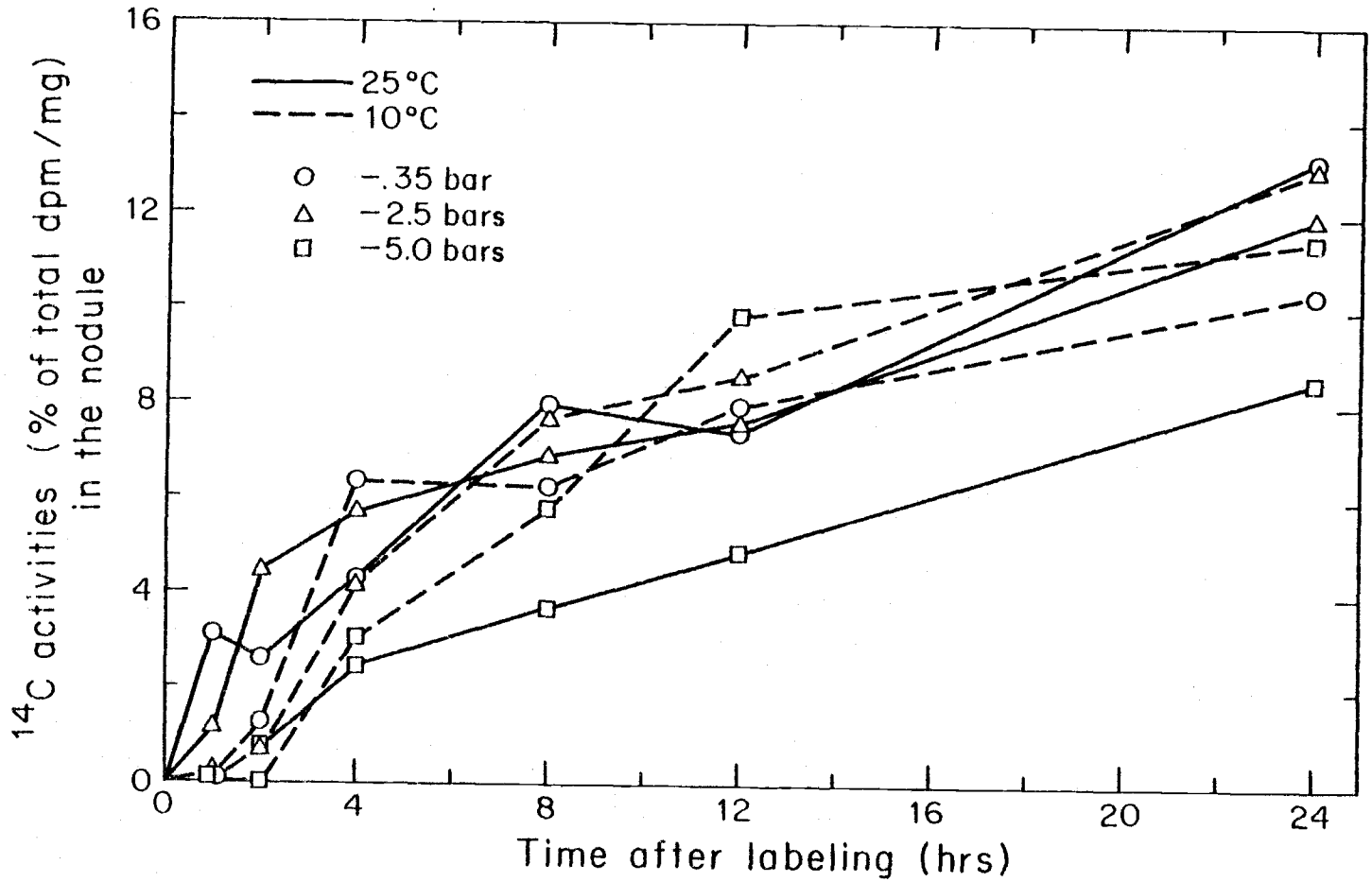

Figure 20. Activities of ^{14}C (percentage of total dpm/mg) in the nodules of soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Table 27. Activities of ^{14}C (% of total dpm/mg) in the nodules of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	----------% of total dpm/mg----------					
25	-0.35	3.14	2.59	4.30	7.94	7.41	13.18
25	-2.5	1.17	4.42	5.74	6.86	7.56	11.93
25	-5.0	0.12	0.75	2.45	3.69	4.85	8.50
10	-0.35	0.07	1.28	6.37	6.22	7.93	13.35
10	-2.5	0.01	0.68	4.20	7.69	8.51	12.97
10	-5.0	0.06	0.04	3.04	5.79	9.79	11.46

$LSD_{.05*} = 1.65$

$LSD_{.01*} = 2.59$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Table 28. Main effects of soil water potential on ^{14}C activities (% of total dpm/mg) in the nodules of 18-day old soybean seedlings at different times after labeling.

Soil water potential	Hours after labeling					
	1	2	4	8	12	24
bar	----------% of total dpm/mg----------					
-0.35	1.61	1.94	5.33	7.08	7.67	13.27
-2.50	0.59	2.55	4.97	7.28	8.03	12.45
-5.00	0.09	0.40	2.75	4.74	7.32	9.98

$LSD_{.05*} = 1.34$

$LSD_{.01*} = 1.83$

*LSD to compare soil water potential means for the same or different times after labeling.

Table 29. Main effect of soil temperature on ^{14}C activities (% of total dpm/mg) in the nodules of 18-day old soybean seedlings at different times after labeling.

Soil temperature	Hours after labeling					
	1	2	4	8	12	24
°C	----------% of total dpm/mg----------					
25.0	1.48	2.59	4.16	6.16	6.61	11.20
10.0	0.07	0.66	4.54	5.65	8.74	12.60

Table 30. Activities of ^{14}C (dpm/mg) in the nodules of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm/mg					
25	-0.35	277	254	403	747	745	1198
25	-2.5	96	365	448	555	614	1018
25	-5.0	10	64	180	303	419	664
10	-0.35	5	98	446	471	536	944
10	-2.5	0	40	249	528	543	806
10	-5.0	3	2	145	307	531	635

$LSD_{.05*} = 201$

$LSD_{.01*} = 276$

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Table 31. Main effects of soil water potential on ^{14}C activities (dpm/mg) in the nodules of 18-day old soybean seedlings at different times after labeling.

Soil water potential	Hours after labeling					
	1	2	4	8	12	24
bar	----------dpm/mg----------					
-0.35	141	176	425	609	641	1071
-2.5	48	203	349	542	578	912
-5.0	7	33	162	305	475	650

$LSD_{.05*}$ = 142

$LSD_{.01*}$ = 195

* LSD to compare soil water potential means for same or different times after labeling.

Table 32. Main effects of soil temperature on ^{14}C activities (dpm/mg) in the nodules of 18-day old soybean seedlings at different times after labeling.

Soil temperature	Hours after labeling					
	1	2	4	8	12	24
°C	----------dpm/mg----------					
25.0	128	228	316	591	632	960
10.0	3	47	280	436	537	795

At 24 hours the nodules at -5.0 bars contained 10 percent of total dpm/mg compared to 13.3 and 12.5 percent of total dpm/mg at -0.35 and -2.5 bars respectively.

There appeared to be no difference in main effects of soil temperature on ^{14}C allocation to the nodules (Table 29). However, when absolute ^{14}C activities in the nodules expressed as dpm/mg (Tables 30, 31 and 32) were considered, the main effects of soil temperature indicated that absolute activities were consistently lower at 10°C than at 25°C. The apparent reasons for the difference in the main effects of soil temperature using the absolute activities (dpm/mg) and percent of total dpm/mg is the reduction in total ^{14}C activities (Table 11) in the plants at 10°C compared to 25°C. The effects of soil temperature on the absolute activities of ^{14}C (dpm/mg) in the nodules may explain the observed reduction in rate of nitrogen fixation at low soil temperatures as reported by Kuo and Boersma (1971), Waughman (1977), and Duke et al. (1979).

Discussion of $^{14}CO_2$ Labeling Study

The time course of ^{14}C activities remaining in the labeled leaves resembles a decay curve. Initially there was a rapid decrease in ^{14}C activities with time followed by a period during which the rate of export decreased gradually (Fig. 16). This pattern has been observed in many plant species (Hofstra and Nelson, 1969). Exportation rate of ^{14}C from the labeled leaves appeared to be affected by soil water potential and soil temperature. At the soil temperature of 25°C the duration of the rapid decline phase lasted about one to two hours after labeling. The rates of decrease in ^{14}C activities during this

phase were 14.2, 8.9 and 3.1 percent total dpm/mg/hr when the soil water potential was at -0.35, -2.5 and -5.0 bars respectively (Table 19).

The soil temperatures of 10°C, the duration of the rapid decline phase was extended to four hours after labeling. Translocation rates during the rapid decline phase at soil temperatures of 10°C were lower than those at 25°C when the same soil water potentials were considered. At the soil temperature of 10°C the exportation rates during the rapid decline phase were 4.7, 3.7 and 2.5 percent of total dpm/mg/hr, at -0.35, -2.5 and -5.0 bars respectively.

The lower ^{14}C activities in the tips of soybeans at lower water potentials (Table 23) runs parallel to the data on effects of soil water potential on elongation rates of the third and fourth trifoliate leaves, which are components of the tips (Table 10). The rates of elongation reflect sink activities and demand for photosynthates. The utilization of carbohydrates for metabolism in the growing tissue steepens the concentration gradient of the phloem solution between source and sink which in turn creates a greater turgor pressure gradient and results in a higher translocation rate according to the pressure flow hypothesis. The results from the labeling studies reported here also agree with the work of Plaut and Reinhold (1965) who demonstrated that a negligible amount of labeled sucrose was transported to the growing tips of bean plants (Phaseolus vulgaris) under water stress.

When the soil water potential was decreased from -0.35 to -2.5 and to -5.0 bars at the soil temperature of 25°C a higher percentage of ^{14}C was translocated to the roots than to the tip (Figs. 17 and 19).

Roots still utilized photosynthates for their growth and activities under water stress which in turn created a concentration gradient necessary for flow of photosynthates from the labeled leaves.

Literature reports of the effects of water stress on ^{14}C distribution to the roots are not consistent. Translocation of photosynthates to the roots of Lolium temulentum L. was less in water stressed than in well watered plants (Wardlaw, 1969). Translocation was less in stressed bean plants initially, but disparity between treatments decreased with time and in the final period (15 hours after labeling) ^{14}C-sucrose in the root was highest in the stressed plant (Plaut and Reinhold, 1965). Activities of ^{14}C (% total dpm) at 48 hours after labeling in the roots of vegetatively growing soybeans was slightly higher in stressed than in the control plants (Silvius et al., 1977). The variation in the observed responses may be due to the differences between plants in growth rates or roots at low soil water potentials. Genetic variability in the growth rates of roots and activities under limited water supply was demonstrated to exist even in the same species (Jordan and Miller, 1980).

At $10^{o}C$, soil water potential treatments had no significant effect on ^{14}C percentages in the roots. This was because the effects of soil water potential were masked by the more pronounced effects of soil temperature on ^{14}C translocation to the roots. Lowering the soil temperature to $10^{o}C$ markedly decreased ^{14}C percentages in the roots and in the tips at the same soil water potential. At this temperature the elongation rate of the expanding leaves were inhibited (Table 10).

Figures 18 and 19 show that at the soil temperature of 10°C, the average rates of increase in percentage of ^{14}C in the stem during the first four hours were 2.2, 1.8 and 1.2 percent of total dpm/mg/hr at -0.35, -2.5, and -5.0 bars respectively while the rates of increase of ^{14}C in the roots during the same period were 0.7, 0.5 and 0.4 percent of total dpm/mg/hr at corresponding soil water potentials. Thus the lower percentage of ^{14}C accumulated in the roots was not caused by the decrease in ^{14}C distributed to the stem at low soil temperature. It was probably caused by the restricted unloading of photosynthates in the roots at soil temperature of 10°C. Effects of root temperature on translocation from shoot to roots of sugar cane were demonstrated (Hartt, 1965).

In a recent review on the effects of temperature on nutritional requirements of plants, Ungs et al. (1982) showed that nutrient uptake is a function of viscosity, membrane permeability, active transport across the root membranes, and growth. The active transport requires metabolic energy for moving nutrients across the plant membranes against an electrochemical potential gradient. The ultimate source of energy utilized for the active transport comes from photosynthates. Thus, a decrease in photosynthate importation by the roots at low soil temperature may cause a reduction in water and nutrient uptake. Dalton and Gardner (1978) demonstrated the importance of active transport on the uptake of solutes by plant roots. Using a model coupling the flow of water and flow of solutes, and accounting for active uptake, their prediction suggested a more rapid drop in solute flux with decreasing soil temperature than predictions using a model without active uptake. Their predicted values were very

similar to the experimental data reported by Kramer (1940). Temperature dependence of active transport, root growth, and photosynthate distribution to the roots and interrelationships among these factors create difficulties differentiating the primary responses of plants to changes in soil temperature. Probably a low soil temperature initiates a decrease in root growth, reduces sink activities which in turn lowers turgor potential in the phloem and translocation to the roots. Decreased availability of photosynthates which are the main sources of metabolic energy for active transport together with high viscosity of water and low permeability of root membranes as a result of low soil temperature will reduce water and nutrient uptake resulting in a reduction in root growth and plant production.

SUMMARY AND CONCLUSIONS

The responses of soybean seedlings to different levels of soil water potential and soil temperature were investigated. Experiments were conducted in the controlled environment of growth chambers. The pressure-volume method was used to determine the parameters which characterize plant water relations and are required for correction of osmotic potential of the leaf sap.

In practice, it was very difficult, if not impossible to prepare fully turgid leaf samples at the beginning of P-V measurements. The method for rehydrating the leaf inside the pressure chamber as reported in the literature was found to be unsuitable for use with soybean leaves. Simple calculation showed that the water content at leaf water potentials ranging from 0 to 2.0 bars was too high to be acceptable. The excess water in the tissue near full turgor is thought to reside in the air spaces near mesophyll cells. Rehydration of the leaf samples by this technique leads to erroneous results. The most serious problems were negative values of the elastic modulus and an increase in turgor potential with decreasing leaf water potential near full turgor. These phenomena can not physically occur. Soybean leaves should be brought to full turgor by submerging the cut petiole in darkness for 12 hours before initiating the P-V measurements. Water content at full turgidity may be estimated using the extrapolation technique as suggested by Ladiges (1975). Other parameters in the range near full turgor may be estimated.

The mean value of osmotic potential of fully expanded soybean leaves at full turgor, estimated from P-V data, was -9.6 ± 0.3 bars.

Mean leaf water potential and relative water content at zero turgor were 13.7 $\pm$ 1.2 bars and 81.3 $\pm$ 6.2 percent respectively. Apoplastic water content ranged from 7.2 to 13.9 percent of total water volume with a mean value of 9.8 $\pm$ 3.0 percent. This average value was used in the calculation of corrected osmotic potentials of the cell sap in the experiments.

The elastic modulus of the tissue decreased with decreasing turgor potential. A graphical procedure outlined by Landsberg (1977) was used to fit ε and Ψ_p data from the P-V measurements to a function of the form $\varepsilon = \varepsilon_m\{1 - \exp(-\Psi_p/k)\}$. The relationship between ε and Ψ_p seems to fit this function. Maximum values of ε estimated from P-V measurements ranged from 76 to 235 bars with an average of 174 $\pm$ 76 bars.

The experiments on the effects of soil water potential and soil temperature on plant water status showed that lowering the soil water potential resulted in a decrease of leaf water potential at both soil temperatures of 25 to 10°C. The decrease in soil water potential from -0.35 to -5.0 bars resulted in a decrease of leaf water potential from -7.3 to -13.5 bars. The leaf water potential was lowered by 2.4 bars when the soil temperature was decreased from 25 to 10°C.

The corrected osmotic potential decreased from -13.8 to -17.8 bars when the soil water potential decreased from -0.35 to -5.0 bars. A decrease in corrected osmotic potential of 1.6 bars occurred when the soil temperature was lowered from 25 to 10°C.

Uncorrected turgor potential decreased from 5.0 to 2.1 bars as a result of changes in leaf water potential and osmotic potential due to the main effects of soil water potential. Corrected turgor

potentials decreased from 6.5 to 4.3 bars, when the soil water potential decreased from -0.35 to -5.0 bars.

The relationship between Ψ_p and Ψ indicated a partial turgor maintenance by osmotic adjustment in the fully expanded soybean leaves. The values of $d\Psi_p/d\Psi$, the slope of the plot of Ψ_p vs Ψ which is linear were 0.48 and 0.37 for the uncorrected and corrected turgor potential data respectively.

An osmotic adjustment index was defined as

$$OI = 1 - \frac{d\Psi_p/d\Psi}{d\Psi_p{}^{v}/d\Psi^{v}}$$

where $d\Psi_p/d\Psi$ and $d\Psi_p{}^{v}/d\Psi^{v}$ are the slopes of plots of turgor potential vs leaf water potential obtained by slowly drying the plants and from the P-V measurements. The OI proposed here was normalized using the reference slope which is based on the no osmotic adjustment condition according to the theory of P-V measurement. It may better serve as an osmotic adjustment index than the one used by Turner and Jones (1980). The OI is useful for comparing the effects of the experimental treatments on degree of osmotic adjustment within and among plant species. It may serve as a tool in breeding and selection programs for plant varieties that possess the osmotic adjustment capability.

There was a significant interaction between soil water potential and soil temperature on elongation rates of the expanding soybean leaves. Elongation rates of the second trifoliate leaves decreased from 11.0 to 1.9 mm/day, whereas those of third and fourth trifoliate leaves decreased from 13.3 and 1.3 mm/day respectively to a zero growth rate at the soil water potentials of 5.0 bars. When the soil temperature was 10°C, elongation rates of the second, third, and fourth

trifoliate leaves were 1.3, 1.9 and 0.3 mm/day respectively at the soil water potential of -0.35 bar. All expanding leaves apparently ceased to elongate at the soil water potential of -2.5 and -5.0 bars.

A $^{14}CO_2$ generating chamber, leaf labeling chamber, and gas flow control system were designed for use in cooperation with the soil water potential and soil temperature control system. Analysis of ^{14}C activities from pulse labeling experiments showed that total ^{14}C activities (dpm) of plants treated at soil water potentials of -2.5 and -5.0 bars were 79 and 67 percent of that at -0.35 bars. Total ^{14}C activities (dpm) in the plants subjected to the soil temperatures of $10^{o}C$ were 70 percent of those grown at the $25^{o}C$ soil temperature treatment.

There was an interaction between soil water potential and soil temperature on ^{14}C activities remaining in the labeled leaves over a 24 hour period. Plants grown at the soil water potential of -0.35 bars retained more ^{14}C activities (% of total dpm/mg) in the labeled first trifoliate leaves than those at -2.5 and -5.0 bars and the soil temperatures of $25^{o}C$. When soil temperature was decreased to $10^{o}C$ there was no significant difference between ^{14}C activities remaining in the labeled leaves of plants at -0.35 and -2.50 bars but the difference was significant between plants at -0.35 and -5.0 bars.

The interaction between soil water potential and soil temperature on ^{14}C activities in the tip was highly significant. At the soil temperatures of $25^{o}C$, ^{14}C activities (% of total dpm/mg) increased at a faster rate in the tips of plants grown at -0.35 bar than of those at -2.5 and -5.0 bars at all hours after labeling. However, when the soil temperature was reduced to $10^{o}C$ the differences between

soil water potential treatments were not significant at any time after labeling, except at 24 hours. Activities of ^{14}C (% of total dpm/mg) in the tips of plants at -0.35 and -2.5 bars were greater than those of plants at -5.0 bars.

The stems of plants growing at -0.35 bar accumulated more ^{14}C activities (% of total dpm/mg) than those at -5.0 bars at all times after labeling. Soil temperature did not appear to affect the amount of ^{14}C in the stem where the same soil water potential levels were compared.

There was a highly significant interaction between soil water potential and soil temperature on ^{14}C activities in the roots. At the soil temperature of 25°C there was a significant difference between percentage of ^{14}C accumulated in the roots at -0.35 and -5.0 bars, but the differences between soil water potential treatments were not significant when plants were grown at the soil temperatures of 10°C. However, ^{14}C activities in the roots of plants grown at soil temperatures of 10°C were lower than those in the roots grown at 25°C at the same soil water potential level.

Differences between percentage of ^{14}C activities in the nodules at -0.35 and -2.50 bars were not significant but ^{14}C activities in the nodules at -5.0 bars were significantly lower than at -0.35 and -2.50 bars. The absolute activities (dpm/mg) in the nodules at 10°C were lower than at 25°C.

General conclusions may be drawn from the results of the experiments reported here. These are tabulated below.

1. The decrease in soil water potential resulted in a decrease in leaf water potential, osmotic potential of tissue sap, and turgor potential of soybean leaves.

2. The decrease in osmotic potential of fully expanded soybean leaves gave rise to partial turgor maintenance of the leaves by osmotic adjustment.

3. The P-V technique is useful for obtaining the apoplastic water content necessary for correcting osmotic potential data determined by the hygrometric method. It is also helpful in characterizing the relationship between parameters describing plant water status. However, the P-V method can not be used to obtain osmotic and turgor potential for plants which have capability of undergoing osmotic adjustment.

4. Elongation of expanding soybean leaves was inhibited at low soil water potential and/or low soil temperature. The magnitude of the decrease in the rate of elongation with decreasing soil water potential depends on the soil temperature. At the soil temperature of $10^{o}C$, there was only a small decrease in rate of leaf elongation with decreasing soil water potential because elongation rate was relatively small even at high soil water potentials.

5. The decrease in soil water potential not only resulted in reducing total amount of ^{14}C fixed in the plant but also decreased percentage of ^{14}C exported from the labeled first trifoliate leaves. This was also true at the soil temperature of $10^{o}C$.

6. The pattern of ^{14}C distribution to different plant parts was altered when the plants were under water stress. Decreasing soil water potential from -0.35 to -5.0 bars resulted in less of the ^{14}C

distributed to all sink sites, but the tips were most severely affected.

7. Interaction between soil water potential and soil temperature treatments on percentage of ^{14}C imported by the growing tips and roots were significant. At the soil temperatures of 25°C, decreasing soil water potential reduced the percentage of ^{14}C in the tips markedly. This effect was less pronounced at soil temperatures of 10°C.

8. Decreasing soil water potential from -0.35 to -5.0 bars significantly decreased percentage of ^{14}C imported by the roots at the soil temperature of 25°C. Differences between soil water potential treatments on percentage of ^{14}C distribution to the roots were not significant at the 10°C soil temperature.

9. Percentage of ^{14}C imported by the nodules was significantly reduced when the soil water potential decreased from -0.35 to -5.0 bars.

10. Lowering the soil temperature from 25 to 10°C resulted in decreased distribution of ^{14}C to the tips and roots. Importation of ^{14}C by the nodules was not affected by the low soil temperature treatment.

11. Rate of importation of ^{14}C by the growing tips seems to be associated with the elongation rate at the corresponding soil water potential and soil temperature treatment.

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APPENDIX

Appendix Table 1. The compositions of a modified Hoagland nutrient solution used for growing soybean.

Nutrient	Nutrient concentration		
	In stock solution		In Hoagland solution
	mole/liter	ppm	mM
K_2SO_4	0.5		1.58
$MgSO_4 \cdot 7H_2O$	1.0		2.00
KH_2PO_4	1.0		0.88
$KHPO_4 \cdot 3H_2O$	1.0		0.14
$CaCl_2$	1.0		6.00
NH_4NO_3	1.0		0.50
$CaSO_4 \cdot 2H_2O$	18.6 g solid		3.57
Fe-EDDHA		1000	2.00
$CoCl_2 \cdot 2H_2O$		360	0.05
H_3BO_3		250	0.25
$MnSO_4 \cdot H_2O$		250	0.25
$ZnSO_4 \cdot 7H_2O$		50	0.05
$CuSO_4 \cdot 5H_2O$		20	0.02
$Na_2MoO_4 \cdot 2H_2O$		10	0.01

Appendix Table 2. ANOVA for leaf water potential of the first trifoliate leaves of soybean as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	36.015	339.408**
Error (a)	6	0.106	
Pot.	2	78.918	401.561**
Temp. x Pot.	2	2.636	13.414**
Error (b)	12	0.197	
Total	23		

** Significance at 1% level.

Appendix Table 3. ANOVA for uncorrected osmotic potential of the first trifoliate leaves of 18-day old soybean seedlings as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	7.820	160.419**
Error (a)	6	0.049	
Pot.	2	21.645	210.320**
Temp. x Pot.	2	2.490	24.198**
Error (b)	12	0.103	
Total	23		

** Significance at 1% level.

Appendix Table 4. ANOVA for corrected osmotic potential of the first trifoliate leaves of 18-day old soybean seedlings as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	13.650	165.182**
Error (a)	6	0.083	
Pot.	2	31.220	164.556**
Temp. x Pot.	2	3.462	18.246**
Error (b)	12	0.190	
Total	23		

** Significance at 1% level.

Appendix Table 5. ANOVA for uncorrected turgor potential of the first trifoliate leaves of 18-day old soybean seedlings as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	10.270	210.675**
Error (a)	6	0.049	
Pot.	2	18.175	84.373**
Temp. x Pot.	2	0.265	1.232
Error (b)	12	0.215	
Total	23		

** Significance at 1% level.

Appendix Table 6. ANOVA for corrected turgor potential of the first trifoliate leaves of 18-day old soybean seedlings as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	5.320	78.018**
Error (a)	6	0.068	
Pot.	2	11.328	27.780**
Temp. x Pot.	2	0.408	0.970
Error (b)	12	0.395	
Total	23		

** Significance at 1% level.

Appendix Table 7. ANOVA for elongation rate (mm/day) of second trifoliate leaves of 18-day old soybean seedlings as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	106.260	55.541**
Error (a)	6	1.913	
Pot.	2	78.323	237.442**
Temp. x Pot.	2	46.448	140.811**
Error (b)	12	0.330	
Total	23		

** Significance at 1% level.

Appendix Table 8. ANOVA for elongation rate (mm/day) of the third trifoliate leaves of 18-day old soybean seedlings as affected by soil temperatures and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	106.260	73.742**
Error (a)	6	1.441	
Pot.	2	135.656	98.411**
Temp. x Pot.	2	77.073	55.912**
Error (b)	12	1.378	
Total	23		

** Significance at 1% level.

Appendix Table 9. ANOVA for elongation rate (mm/day) of the fourth trifoliate leaves of 18-day old soybean seedlings as affected by soil temperature and soil water potential.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	0.080	15.783**
Error (a)	6	1.260	
Pot.	2	2.344	29.348**
Temp. x Pot.	2	1.260	15.783**
Error (b)	12	0.080	
Total	23		

** Significance at 1% level.

Appendix Table 10. ANOVA for total ^{14}C activities (dpm) in 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-10}	
Temp.	1	525.224	105.860**
Error (a)	4	4.962	
Pot.	2	234.876	218.371**
Temp. x Pot.	2	1.443	1.341
Error (b)	8	1.076	
Time	5	0.861	0.507
Temp. x Time	5	1.500	0.883
Pot. x Time	10	1.756	1.033
Temp. x Pot. x Time	10	1.318	0.776
Error (c)	60	1.699	
Total	107		

** Significance at 1% level.

Appendix Table 11. ANOVA for total ^{14}C activities (dpm/mg) in the whole plant of 18-day old seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-6}	
Temp.	1	139.862	467.590**
Error (a)	4	0.299	
Pot.	2	20.245	29.598**
Temp. x Pot.	2	3.226	4.716*
Error (b)	8	0.684	
Time	5	4.201	1.281
Temp. x Time	5	2.463	0.751
Pot. x Time	10	2.906	0.886
Temp. x Pot. x Time	10	5.522	1.683
Error (c)	60	3.280	
Total	107		

** Significance at 1% level.

* Significance at 5% level.

Appendix Table 12. ANOVA for ^{14}C activities (dpm/mg) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		$\times 10^{-6}$	
Temp.	1	41.354	62.722**
Error (a)	4	0.659	
Pot.	2	1.199	2.516
Temp. x Pot.	2	8.796	18.452**
Error (b)	8	0.477	
Time	5	19.884	54.287**
Temp. x Time	5	1.354	3.696**
Pot. x Time	10	0.553	1.509
Temp. x Pot. x Time	10	0.618	1.688
Error (c)	60	0.366	
Total	107		

** Significance at 1% level.

Appendix Table 13. ANOVA for ^{14}C activities (% total dpm) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	2309.080	44.830**
Error (a)	4	51.507	
Pot.	2	993.346	100.843**
Temp. x Pot.	2	167.463	17.001**
Error (b)	8	9.850	
Time	5	2878.830	175.895**
Temp. x Time	5	110.516	6.752**
Pot. x Time	10	21.060	1.287
Temp. x Pot. x Time	10	20.843	1.274
Error (c)	60	16.367	
Total	107		

** Significance at 1% level.

Appendix Table 14. ANOVA for ^{14}C activities (% total dpm/mg) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	1539.45	43.70**
Error (a)	4	35.23	
Pot.	2	1272.13	189.36**
Temp. x Pot.	2	189.63	28.23**
Error (b)	8	6.72	
Time	5	3227.71	225.98**
Temp. x Time	5	66.46	4.65**
Pot. x Time	10	21.50	1.51
Temp. x Pot. x Time	10	22.06	1.54
Error (c)	60	14.28	
Total	107		

** Significance at 1% level.

Appendix Table 15. ANOVA for ^{14}C activities (% of total dpm/mg) in the second trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	3.463	13.894*
Error (a)	4	0.249	
Pot.	2	0.840	5.285*
Temp. x Pot.	2	0.534	3.361
Error (b)	8	0.159	
Time	5	1.896	15.437**
Temp. x Time	5	1.048	8.531**
Pot. x Time	10	0.197	1.605
Temp. x Pot. x Time	10	0.203	1.651
Error (c)	60	0.123	
Total	107		

*Significant at 5% level.

**Significant at 1% level.

Appendix Table 16. ANOVA for ^{14}C activities (% of dpm/mg) in the tips of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	219.279	46.985**
Error (a)	4	4.667	
Pot.	2	156.188	56.830**
Temp. x Pot.	2	73.587	26.775**
Error (b)	8	2.748	
Time	5	97.456	51.079**
Temp. x Time	5	17.524	9.185**
Pot. x Time	10	8.544	4.478**
Temp. x Pot. x Time	10	4.005	2.099*
Error (c)	60	1.908	
Total	107		

*Significant at 5% level.

**Significant at 1% level.

Appendix Table 17. ANOVA for ^{14}C activities (% of total dpm/mg) in the stems of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	26.127	1.666
Error (a)	4	15.679	
Pot.	2	117.281	82.530**
Temp. x Pot.	2	11.067	7.787*
Error (b)	8	1.421	
Time	5	237.314	111.317**
Temp. x Time	5	8.402	3.941*
Pot. x Time	10	3.236	1.518
Temp. x Pot. x Time	10	3.959	1.857
Error (c)	60	2.132	
Total	107		

*Significant at 5% level.

**Significant at 1% level.

Appendix Table 18. ANOVA for ^{14}C activities (% of total dpm/mg) in the roots of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	335.598	153.022**
Error (a)	4	2.193	
Pot.	2	40.886	46.386**
Temp. x Pot.	2	21.130	23.973**
Error (b)	8	0.881	
Time	5	164.817	133.757**
Temp. x Time	5	13.554	11.000**
Pot. x Time	10	0.989	0.627
Temp. x Pot. x Time	10	1.459	0.320
Error (c)	60	1.232	
Total	107		

** Significance at 1% level.

Appendix Table 19. ANOVA for ^{14}C activities (% of total dpm/mg) in the nodules of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
Temp.	1	0.682	0.349
Error (a)	4	1.954	
Pot.	2	41.357	10.083**
Temp. x Pot.	2	14.746	3.595
Error (b)	8	4.102	
Time	5	306.617	143.216**
Temp. x Time	5	11.178	5.221**
Pot. x Time	10	2.325	1.086
Temp. x Pot. x Time	10	3.034	1.417
Error (c)	60	2.141	
Total	107		

** Significance at 1% level.

Appendix Table 20. Activities of ^{14}C (dpm/mg) in the second trifoliate leaves of 18-day old seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm/mg					
25	-0.35	9	11	37	105	142	144
25	-2.5	6	4	18	42	69	146
25	-5.0	2	2	3	15	19	85
10	-0.35	4	5	5	14	18	31
10	-2.5	2	2	2	71	8	12
10	-5.0	2	9	15	2	5	10

$LSD_{.05}$* = 43

$LSD_{.01}$* = 58

* LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 21. Activities of ^{14}C (dpm/mg) in the tips of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm/mg					
25	-0.35	243	333	466	957	1287	1344
25	-2.5	34	114	124	253	407	693
25	-5.0	4	15	31	72	86	468
10	-0.35	35	60	63	200	182	348
10	-2.5	8	16	81	102	144	307
10	-5.0	2	5	16	21	27	120

$LSD_{.05}$* = 209

$LSD_{.01}$* = 284

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 22. Activities of ^{14}C (dpm/mg) in the stems of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time of labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm/mg					
25	-0.35	576	613	644	828	1223	1402
25	-2.5	351	556	605	744	883	1139
25	-5.0	97	317	415	443	633	1194
10	-0.35	249	467	617	877	840	1038
10	-2.5	141	264	447	506	487	709
10	-5.0	25	152	263	332	406	546

$LSD_{.05}$* = 171

$LSD_{.01}$* = 230

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 23. Activities of ^{14}C (dpm/mg) in the roots of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
^{o}C	bar	dpm/mg					
25	-0.35	150	506	551	617	945	1149
25	-2.5	128	427	545	711	777	1136
25	-5.0	11	124	239	302	445	896
10	-0.35	11	73	189	314	323	457
10	-2.5	6	29	113	219	252	392
10	-5.0	6	6	78	144	225	296

$LSD_{.05}^{*} = 132$

$LSD_{.01}^{*} = 178$

* LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 24. Activities of ^{14}C (dpm) remaining in the source leaves of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
^{o}C	bar	dpm x 10^{-3}					
25	-0.35	1380	1361	1440	1226	1005	683
25	-2.5	1211	1019	852	1139	1179	949
25	-5.0	1257	1109	1070	1089	1061	720
10	-0.35	1311	1204	1121	1026	980	843
10	-2.5	1139	867	862	833	810	648
10	-5.0	708	764	747	629	657	620

$LSD_{.05}$* = 224

$LSD_{.01}$* = 301

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 25. Activities of ^{14}C (dpm) in the second trifoliate leaves of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm					
25	-0.35	1914	2149	7454	22797	25760	34482
25	-2.5	2160	4837	8944	4265	5894	13273
25	-5.0	254	292	465	1918	2647	12448
10	-0.35	537	628	598	1885	2455	4644
10	-2.5	256	240	208	7026	1052	1653
10	-5.0	282	945	1418	245	555	1297
	$LSD_{.05}$* = 8264						
	$LSD_{.01}$* = 11178						

* LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 26. Activities of ^{14}C (dpm) in the tips of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm					
25	-0.35	28891	46692	59420	134340	153392	232422
25	-2.5	10107	21015	32748	15996	26590	40688
25	-5.0	380	1369	3537	7569	7837	50511
10	-0.35	4088	5453	6293	20366	18596	37963
10	-2.5	795	1575	8028	8289	12574	31884
10	-5.0	171	436	1250	1753	2211	11898

$LSD_{.05}$* = 26358

$LSD_{.01}$* = 35629

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 27. Activities of ^{14}C (dpm) in the stems of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
^{o}C	bar	dpm x 10^{-3}					
25	-0.35	145	172	175	222	286	382
25	-2.5	122	166	201	135	179	234
25	-5.0	22	71	105	108	151	302
10	-0.35	59	113	152	210	210	276
10	-2.5	33	62	110	109	120	173
10	-5.0	6	31	59	77	94	138

$LSD_{.05}$* = 66

$LSD_{.01}$* = 88

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 28. Activities of ^{14}C (dpm) in the roots of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm x 10^{-3}					
25	-0.35	41	154	176	188	276	386
25	-2.5	79	144	210	128	161	240
25	-5.0	2	31	59	73	103	234
10	-0.35	2	16	46	64	75	113
10	-2.5	1	6	28	40	63	90
10	-5.0	1	1	16	28	46	69

$LSD_{.05}$* = 68

$LSD_{.01}$* = 90

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 29. Activities of ^{14}C (dpm) in the nodules of 18-day old soybean seedlings as a function of soil water potential, soil temperature, and time after labeling.

Soil temperature	Soil water potential	Hours after labeling					
		1	2	4	8	12	24
°C	bar	dpm x 10^{-3}					
25	-0.35	5782	4307	7229	15236	13465	23588
25	-2.5	4045	8529	11886	7527	10810	14118
25	-5.0	239	878	3201	6395	6505	14070
10	-0.35	112	2844	10391	11073	14536	27978
10	-2.5	11	946	6603	11005	15018	17965
10	-5.0	64	46	3775	7086	12123	17949

$LSD_{.05}$* = 6015

$LSD_{.01}$* = 8194

*LSD to compare soil water potential means for the same soil temperature and same or different times after labeling.

Appendix Table 30. ANOVA for the ^{14}C activities (dpm/mg) in the second trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-3}	
Temp.	1	34.169	24.388**
Error (a)	4	1.401	
Pot.	2	7.946	10.516**
Temp. x Pot.	2	5.403	7.150*
Error (b)	8	0.756	
Time	5	12.930	20.719**
Temp. x Time	5	8.147	13.055**
Pot. x Time	10	1.378	2.208*
Temp. x Pot. x Time	10	1.395	2.235*
Error (c)	60	0.624	
Total	107		

*Significance at 5% level.
**Significance at 1% level.

Appendix Table 31. ANOVA for ^{14}C activities (dpm/mg) in the tips of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-5}	
Temp.	1	22.476	76.175**
Error (a)	4	0.295	
Pot.	2	14.207	73.988**
Temp. x Pot.	2	7.728	40.246**
Error (b)	8	0.192	
Time	5	6.370	43.670**
Temp. x Time	5	1.865	12.789**
Pot. x Time	10	0.807	5.533**
Temp. x Pot. x Time	10	0.444	3.044**
Error (c)	60	0.146	
Total	107		

** Significance at 1% level.

Appendix Table 32. ANOVA for ^{14}C activities (dpm/mg) in the stems of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-4}	
Temp.	1	153.797	28.811**
Error (a)	4	5.338	
Pot.	2	130.115	174.486**
Temp. x Pot.	2	1.782	2.390
Error (b)	8	0.746	
Time	5	131.763	117.598**
Temp. x Time	5	9.530	8.506**
Pot. x Time	10	2.078	1.855
Temp. x Pot. x Time	10	2.034	1.815
Error (c)	60	1.120	
Total	107		

** Significance at 1% level.

Appendix Table 33. ANOVA for ^{14}C activities (dpm/mg) in the roots of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-4}	
Temp.	1	354.616	329.021**
Error (a)	4	1.078	
Pot.	2	43.650	94.737**
Temp. x Pot.	2	15.831	34.359**
Error (b)	8	0.461	
Time	5	99.625	149.968**
Temp. x Time	5	17.121	25.773**
Pot. x Time	10	1.093	1.645
Temp. x Pot. x Time	10	0.813	1.224
Error (c)	60	0.664	
Total	107		

** Significance at 1% level.

Appendix Table 34. ANOVA for ^{14}C activities (dpm) remaining in the labeled first trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-10}	
Temp.	1	131.971	19.160**
Error (a)	4	6.888	
Pot.	2	64.137	66.061**
Temp. x Pot.	2	15.726	16.197**
Error (b)	8	0.971	
Time	5	35.486	17.678**
Temp. x Time	5	2.909	1.449
Pot. x Time	10	5.658	2.819**
Temp. x Pot. x Time	10	4.014	2.000*
Error (c)	60	2.007	
Total	107		

*Significance at 5% level.
**Significance at 1% level.

Appendix Table 35. ANOVA for ^{14}C activities (dpm) in the second trifoliate leaves of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-8}	
Temp.	1	13.236	33.980**
Error (a)	4	0.390	
Pot.	2	4.427	19.074**
Temp. x Pot.	2	3.429	14.776**
Error (b)	8	0.232	
Time	5	2.725	11.047**
Temp. x Time	5	1.683	6.821**
Pot. x Time	10	0.722	2.927**
Temp. x Pot. x Time	10	0.632	2.563*
Error (c)	60	0.247	
Total	107		

*Significance at 5% level.

**Significance at 1% level.

Appendix Table 36. ANOVA for ^{14}C activities (dpm) in the tips of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-8}	
Temp.	1	408.194	105.705**
Error (a)	4	3.862	
Pot.	2	307.520	134.489**
Temp. x Pot.	2	203.661	89.068**
Error (b)	8	2.287	
Time	5	86.182	34.107**
Temp. x Time	5	28.367	11.227**
Pot. x Time	10	26.839	10.622**
Temp. x Pot. Time	10	19.891	7.872**
Error (c)	60	2.527	
Total	107		

** Significance at 1% level.

Appendix Table 37. ANOVA for ^{14}C activities (dpm) in the stems of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-9}	
Temp.	1	109.357	53.307**
Error (a)	4	2.051	
Pot.	2	96.902	135.781**
Temp. x Pot.	2	0.410	0.575
Error (b)	8	0.714	
Time	5	73.489	41.472**
Temp. x Time	5	3.557	2.008
Pot. x Time	10	1.772	2.194*
Temp. x Pot. x Time	10	3.887	1.108
Error (c)	60	1.963	
Total	107		

*Significance at 5% level.

**Significance at 1% level.

Appendix Table 38. ANOVA for ^{14}C activities (dpm) in the roots of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-9}	
Temp.	1	325.922	1004.486**
Error (a)	4	0.324	
Pot.	2	48.373	114.255**
Temp. x Pot.	2	20.926	49.426**
Error (b)	8	0.423	
Time	5	58.756	30.207**
Temp. x Time	5	11.923	6.130**
Pot. x Time	10	3.101	1.594
Temp. x Pot. x Time	10	2.392	1.230
Error (c)	60	1.945	
Total	107		

** Significance at 1% level.

Appendix Table 39. ANOVA for ^{14}C activities (dpm) in the nodules of 18-day old soybean seedlings as a function of soil temperature, soil water potential, and time after labeling.

Source of variation	d.f.	Mean square	Observed F
		x 10^{-6}	
Temp.	1	0.245	0.040
Error (a)	4	6.063	
Pot.	2	259.043	14.858**
Temp. x Pot.	2	16.268	0.933
Error (b)	8	17.435	
Time	5	751.661	64.185**
Temp. x Time	5	46.260	3.9501**
Pot. x Time	10	26.656	2.276*
Temp. x Pot. x Time	10	15.062	1.286
Error (c)	60	11.711	
Total	107		

*Significance at 5% level.
**Significance at 1% level.