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MANIPULATION OF SINK SIZE AND THE DYNAMICS OF PHOTOSYNTHATE TRANSLOCATION IN PHASEOLUS VULGARIS L.

by

George Ibrahim Ghobrial

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF BIOLOGICAL SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN BOTANY

In the Graduate College

THE UNIVERSITY OF ARIZONA

1973
I hereby recommend that this dissertation prepared under my direction by George Ibrahim Ghobrial entitled MANIPULATION OF SINK SIZE AND THE DYNAMICS OF PHOTOSYNTHATE TRANSLOCATION IN PHASEOLUS VULGARIS L. be accepted as fulfilling the dissertation requirement of the degree of DOCTOR OF PHILOSOPHY.

James W. O'Leary
Dissertation Director
Dec. 3, 1973

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:

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ABSTRACT

The influence of manipulating root size, a major sink for photosynthate, on the translocation dynamics of assimilate, as well as on the growth and partitioning of the dry matter in the different parts of Red Kidney bean (*Phaseolus vulgaris* L.) plants was studied. The plants were grown in aerated nutrient solutions in controlled environmental conditions. The effect of manipulation of root size on some growth parameters and on partitioning of dry matter were investigated in plants grown under various carbon dioxide concentrations, relative humidities, and night temperatures. In simplified plants, pruned to one mature source leaf and two opposite sinks the influence of root size was demonstrated in regard to the level of soluble sugar content, translocation velocity of assimilated C-14 toward the two opposite sinks, and on the distribution of the assimilated C-14 in the different plant parts, including the manipulated sink itself. Influence of partial derootment on the distribution pattern of assimilated C-14 in complete plants was also investigated.

The effect of removing 50 per cent of the roots on the growth of plants was variable, depending on the conditions of each trial. While the partial derootment increased growth of shoots and roots of plants grown under high relative humidity, it depressed the growth of shoots, and, to a lesser degree, roots of plants grown under higher than ambient concentration of carbon dioxide and low relative humidity. Although the partial derootment did not affect the total export of
assimilated C-14 from the source leaf, it did alter the distribution pattern, with less moved to the manipulated sink itself. These effects follow closely the supply and demand concept. The manipulated sink in this study was the root, which was also a source for some growth hormones required by the shoot. Thus, removing 50 percent of the roots decreased the mobilization power of the shoots by depriving them of these hormones. Consequently, the translocation velocity as well as the distributed C-14, was reduced toward these parts. The partial derootment increased the level of soluble sugars in the different plant parts. However, their concentration gradient down the stem was not changed, nor was the velocity toward the roots. The results suggested that roots of Red Kidney bean plants influence translocation of assimilates in two ways. First, they are a major sink for the assimilate, and second, they are a source of some growth hormones required to maintain the mobilization power of the shoot parts and consequently translocation of assimilates toward them. Partial derootment increased the availability of photosynthate in the whole plant but it reduced the capacity of their utilization. The final distribution pattern of photosynthate, therefore, was the net result of these two interrelated processes, leading to an accumulation in the lower stem parts. Spraying the partially derooted plants with Kinetin increased the capacity for utilization of assimilate.

It seems, therefore, that the alteration in translocation dynamics resulting from perturbation such as reducing root size is associated with the survival value attached to maintaining a balance between root and shoot size,
INTRODUCTION

One of the main goals in studying the physiology of plants is to make clear the relationships between structure and function. This could be on a molecular, cellular, or organismic level. It is unique to translocation physiology, however, that the structures involved are in continuous connection through all the plant organs having different structures and functions. For this reason, physiology of translocation can be considered as a higher level in plant physiology; it involves the physiology of the whole plant.

Since availability of photosynthate as well as the efficiency of its translocation will determine the yielding capacity of plants, realizing the limits and the extents of the translocation process may enable man to direct translocation toward what he needs from plants. Generally, translocation of photosynthate is ruled by the law of supply and demand. Manipulation of the demand by a known sink is likely to change the supply of photosynthate to other sinks. This research project was undertaken to determine the effect of manipulation of sink size, specifically the roots, on the dynamics of photosynthate translocation. The objective was to elucidate the limits and the extents of manipulation of the sink size on the supply and translocation of photosynthate to other sinks in the plant and to the manipulated sink as well.
REVIEW OF LITERATURE

General

Substances move freely from one place to another along their concentration gradients, by a simple diffusion process, as long as there is no barrier to restrict their movement. This process of diffusion seems to be of sufficient magnitude for translocation of the metabolites within the cell, and, therefore, for unicellular organisms diffusion plays a significant role for translocation and absorption of most of their metabolites and waste products from and to their surroundings. But at a higher level of organization and specialization of functions such as found in higher plants, where the leaves carry on photosynthesis far away from the roots which absorb water and nutrients from the soil, and a rapid distribution of the metabolic products of each organ is necessary for the survival of the whole plant, diffusion becomes of negligible importance.

Long-distance transport is usually of great magnitude, and specialized tissues, xylem and phloem, are involved. Generally, the xylem carries water and inorganic nutrients upward from the roots to the leaves, while the phloem carries the organic solutes, products of photosynthesis, mainly downward from the leaves to the roots. The term translocation has been used very often for the phloem transportation but other terms such as descending flow (Kursanov 1961) have also been used.
Swanson (1959) described translocation as a transport process which is most directly associated with specific transport tissues, mainly phloem, and is characterized by considerably high rates (potential or capacity) as well as by movement over great distance from organ to organ. Zimmermann (1969) characterized translocation by the following:

1. Phloem transport, typically, takes place between the following centers

   ![Diagram of phloem transport centers]

   - Photosynthetic tissues (mainly leaves)
   - All non-green plant parts requiring food for metabolism and growth (meristematic tissues, flowers, fruits, etc.)
   - Storage centers in leaves and axes (stem and roots, wood and bark, etc.)

2. Large amounts of substances move over long distance.

3. Special organization of the conducting tissues.

The transport of substances in a specialized conducting system such as phloem seems more economical as regards expenditure of energy per unit length of path covered (Kursanov 1963).

**Nature of Translocated Substances in the Phloem**

More than forty years ago Mason and Maskell (1928), in their classical work on transport of carbohydrates in the cotton plant, examined the radial distribution of sugar in the bark of cotton plants. They found sucrose increased in concentration from outside to inside. Because of the high positive correlation between the percentage of
sieve tubes in any zone in the bark and the sucrose concentration in that specific zone, they concluded that sucrose is the major sugar form translocated in the phloem of cotton plants.

The availability of radioisotopes and more refined techniques of chromatography has opened the door widely for more detailed studies on the nature of photosynthates and the translocated compounds. Vernon and Aronoff (1952), using soybeans and radioactive carbon dioxide, detected the radioactivity in the compounds extracted from the leaves and stems. Within 20 minutes after exposure of the plants to radioactive carbon dioxide, the following radioactive compounds in order of decreasing radioactivity were found in the leaf extract: sucrose, alanine, glutamic acid, glyceric acid, glucose, raffinose, fructose, malic acid, triose, isocitric acid, succinic acid, aspartic acid, and citric acid. Stem sections revealed activity only in sucrose, glucose, and fructose. Therefore it seems not all photosynthates are readily available for translocation.

Sucrose was found to be the major exported metabolite from the leaves of beans (Biddulph and Cory 1957, 1965; Weidner 1964). Glucose and fructose were also present in minute amounts in the translocation stream. Jones, Martin, and Porter (1959) studied translocation of C-14 in tobacco following assimilation of radioactive carbon dioxide by a single leaf. They found 90 per cent of the activity in the stem pieces was in sucrose. Other workers (Shiroya et al. 1961) using tobacco plants recovered 80-100 per cent of the radioactivity as sucrose. They concluded that sucrose is the main form in which carbon was translocated in tobacco. Using Curcurbita pepo, Kursanov (1957)
reported that the downgoing mixture consisted chiefly of sucrose. Organic acids and amino acids made up an insignificant portion of the downflow,

Swanson and El-Shishiny (1958) showed that sucrose is the only form of sugar which is rapidly translocated in the grape plant. Because the ratio of glucose C-14 to fructose C-14 in the extract was essentially unity, they concluded that glucose and fructose are hydrolytic products of the translocatory sucrose.

Sucrose is the major translocated compound in sugarbeet (Turkina 1959). Mortimer (1965) studied the quantity and kinds of radioactive compounds in the blade, mid rib, and petiole of sugarbeet leaves at intervals up to 3 hours. After 8 minutes, radioactive sucrose from the blade reached the rib and began to move down the petiole. He reported that sucrose was the dominant labeled substance even after one minute. The proportion of C-14 in sucrose increased rapidly with time until after ten minutes more than 90 per cent of the total in the petiole was recovered in sucrose. Geiger and Swanson (1965) also demonstrated that sucrose is the major precursor of the translocate in sugarbeets. More evidence that sucrose is the dominant translocated substance came from the work of Clauss et al. (1959) and Nelson et al. (1961) on soybean, and Ahrens and Reid (1973) on pine seedlings.

Sucrose seems to have unique properties that make it a suitable substance for translocation in many diverse species. Although the exact nature of such properties is not known, it appears that sucrose is less reactive than many other sugars, therefore sucrose acts as a protected
derivative of glucose, and thus it has evolved as the preferred form of carbohydrate for translocation. Sucrose is much less reactive, and is thus able to move over long distances and to deliver its glucose residues to sites of growth or storage (Arnold 1968).

Although sucrose is the form of translocated carbohydrate in most of the plant species studied, other forms of oligosaccharides have been shown to play a role similar to sucrose in a few species. Zimmermann (1957) found in some tree species most (white ash) or part (elm, linder) of the sieve tube sugars are in the form of raffinose and stachyose. In straight-necked squash, Webb and Gorham (1964) found that photosynthetically assimilated C-14 appeared in sucrose, stachyose, and raffinose. Weidner (1964) reported that stachyose plays a similar role as sucrose in cucumber and white ash. Other compounds such as steroids were also detected in the translocation stream but in minute amounts (Biddulph and Corry 1965). The composition of the translocated solution may vary with the stage of growth (Kursanov 1961). In soybeans, Nelson et al. (1961) showed that the proportion of C-14 in serine decreased more steadily with age of plants.

Apparently when sugars reach sites of growth, especially the root, they are rapidly converted into compounds of an ionic character such as organic acids and amino acids (Kursanov 1957). Part of the translocated sucrose seems to be used in respiration of the conducting elements (Turkina 1959; Canny 1960).

In summary, sucrose is the dominant form of translocatable carbohydrate in many plant species belonging to very diverse families.
Other forms of oligosaccharides, such as raffinose and stachyose, play a similar role to sucrose in a few species.

**Translocation Pathway**

Translocation begins with formation of sugars and other photosynthates in the chlorophyllous mesophyll cells of the leaves, which then successively pass through a series of parenchyma cells to the nearest vascular bundle. This step is recognized as vein loading, and seems to require metabolic energy (Barrier and Loomis 1957, Plaut and Reinhold 1969). Kursanov and Brovchenko (1961) suggested that phosphorylation of sugars precedes their transfer from the mesophyll cells to the phloem. They noticed activation by ATP increased the rate of outflow of assimilate from the leaf blade of sugar beets. Recent evidence (Sovonick and Geiger 1973) supports the conclusion that the loading step is an active process, because it has been shown that the mesophyll cells of sugar beets have a lower osmotic pressure than the vascular tissue. This ruled out a strictly diffusional movement from the mesophyll cells into the phloem.

Vein loading, the step preceding translocation (Geiger and Cataldo 1969) takes place only after leaf tissues reach a certain growth stage (Leonard and King 1968). Trip (1969), by means of autoradiography, demonstrated visually the process of assimilation by the green cells, collection of sugar in the minor veins, and export in phloem elements. Trip suggested that in minor veins sugar is translocated in companion cells rather than sieve tubes. In major veins translocation occurs in sieve tubes. The companion cells of minor
veins seem to accumulate carbohydrates from the surrounding tissues and to secrete them laterally to sieve tubes, lying alongside the companion cells for further transport along the minor veins.

Following the step of vein loading, translocation proceeds mainly in the phloem. This tissue, and more specifically the sieve elements, comprises the major translocation pathway for assimilates. Support came from ringing experiments. Mason and Maskell (1928) demonstrated that ringing caused a marked accumulation of sugars in both wood and bark above the ring and marked decline below it. They concluded that the longitudinal transport of sugars takes place mainly in the sieve tubes. Using soybeans, Perkins, Nelson, and Gorham (1959) used autoradiography to identify the phloem as the tissue involved in the downward translocation of C-14 labeled photosynthates. Mortimer (1965), using radioactive carbon dioxide on sugarbeets, demonstrated that radioactive sucrose was confined to the phloem region of the vascular bundle.

The basic components of the phloem are the sieve elements, companion cells, parenchyma cells and fibers (Esau 1967). It is agreed that the specialized sieve elements of the phloem provide the channel for translocation (MacRobbie 1971). The fraction of the phloem which is occupied by sieve tubes has commonly been taken to be only 20 per cent. Sieve tube elements are typically 20-30 μm in diameter and 100-500 μm long. They show deficiencies and degeneracy in the normal cellular organelles but have a characteristic proteinaceous content, named P, protein (Cronshaw and Esau 1968a, 1968b). Functions of P, protein are not known but it has been suggested that it may be involved
in production of the motive force and it may have a function in blockage of the sieve pores in damaged sieve elements.

Despite the fact that phloem is the main tissue involved in longitudinal translocation, lateral movement from the phloem to the xylem has been reported by Biddulph, Biddulph, and Cory (1958) and Biddulph and Cory (1965). Clor, Crafts, and Yamaguchi (1963) reported that under certain conditions, such as high relative humidity, translocation is stimulated in both phloem and xylem. Nelson, Perkins, and Gorham (1959) demonstrated that part of the radioactive carbon was translocated through a steam girdled soybean stem. Their conclusion was that translocation could also take place in the xylem. While recognizing the possibility of participation of the xylem in translocation of assimilates, it is clear that translocation of the major compounds such as sugars takes place only in the phloem and more specifically in the sieve tubes.

**Kinetics of Translocation**

The most striking feature of the translocation process is the enormous rate of mass transfer which is defined as dry weight transfer per hour per square centimeter of phloem. Canny (1971) reviewed the kinetics of translocation and reported an average value of 4 grams for mass transfer. If the transfer of dry matter occurred as flow of a solution of organic compounds through sieve tubes, the mass transfer would equal the concentration of solution times the translocation velocity:
Mass Transfer = Concentration of Solution x velocity

\[ \text{g hr}^{-1} \text{ cm}^{-2} \times \text{g cm}^{-3} \times \text{cm hr}^{-1} \]

The concentration of sucrose in the phloem ranges from 5-25% and a maximum mass transfer of 6 g hr\(^{-1}\) cm\(^{-2}\) has been reported (Canny 1971). Therefore, the speed of translocation varies greatly between individual plants and plant parts.

Movement of sucrose or closely related polymers occurs down a gradient of sucrose concentration (Mason and Maskell 1928). In steady state the factor that seems to control the amount of mass transfer and the direction in which dry weight transfer occurs is the gradient of sucrose concentration in the phloem. The value of mass transfer is directly proportional to this gradient (Mason and Maskell 1928, Canny 1971).

\[ M = K \frac{ds}{dx} \]

where \( M = \) the mass transfer

\[ K = \text{the translocation coefficient} \]

\[ s = \text{the sucrose concentration} \]

and \( dx = \) the distance.

Canny reported that this quantitative explanation is adequate for values of \( M \) up to 6 and distances of 10 cm or so or for smaller values of \( M \) over larger distances.

Radioisotopes helped to a great extent in measurement of translocation velocity. The technique involves following the distribution of radioactivity concentration vs. distance from the source. This gives the "profile" of the advancing isotope, from which one can obtain the
distance the radioisotope has traveled per unit time. But actually what we measure here is the apparent velocity which is a function of the level of detection and the amount of radioactivity introduced (Trip and Gorham 1968). Using this technique is also helpful for measuring the relative translocation rates because differences in slopes of profiles indicate differences in the rate of translocation (Nelson et al. 1961). However, this should be used with some caution since the slope is not only a function of translocation rate but also is a function of rate of formation of photosynthates and rate of entrance of photosynthate to sieve tubes (Vernon and Aronoff 1952).

Many translocation physiologists have measured the translocation velocity of C-14 assimilates using the approach of the radioactivity profile. Biddulph and Cory (1957) on Phaseolus vulgaris reported a velocity of 107 cm hr\(^{-1}\). On soybeans, a velocity of 84 cm hr\(^{-1}\) was reported by Vernon and Aronoff (1952). Mortimer (1965) found the velocity in sugarbeets ranges from 50 to 135 cm hr\(^{-1}\). An extremely high value for velocity, over 5000 cm hr\(^{-1}\) was reported by Nelson, Perkins, and Gorham (1959) on soybeans. This velocity was 50 times greater than the velocity at which sucrose is translocated in the phloem. Although the general method for measuring the velocity of translocation is by advancing radioactivity profile, Canny (1961) developed a different approach. Briefly, if one measures the rate of change of radioactivity with time at a place on a plant organ carrying radioactive tracer, and then dissects and extracts the organ and estimates the fall of radioactivity with distance, the former slope divided by the latter gives the velocity of advance of the radioactive
profile. Canny reported velocities in the neighborhood of $2 \text{ cm/hr}^{-1}$ in willow plants.

It is of some interest to note that the radioactivity profile followed a logarithmic relation, i.e., a plot of log radioactivity vs. distance gives a linear relationship (Vernon and Aronoff 1952; Horwitz 1958; Canny 1962; Clauss, Mortimer, and Gorham 1964). It appears that logarithmic gradient is a result of leakage of assimilate C-14 from the translocation channel (Canny 1962). However, Mortimer (1965) found the concentration of C-14 assimilate in sugarbeets was a linear function of the distance. He reasoned that there are some differences in the mode of translocation between sugarbeets and other species, such as soybeans, which produce a logarithmic gradient.

In an extensive study of translocation kinetics in soybeans, Fisher (1970a, 1970b, 1970c) concluded that the kinetics of C-14 translocation must be determined primarily by factors operating within the leaf rather than the stem. This conclusion supports findings of Hartt, Kortschak, and Burr (1964) that the driving force of translocation is within the leaf itself. The specific activity of leaf sucrose reached a maximum within five minutes after pulse labeling whereas that of exported sucrose did not reach a maximum until after twenty minutes. Fisher concluded this was due to the presence of two sucrose compartments in the leaf.

Source/Sink Relations in Translocation

Sources are regions that produce assimilates, by photosynthesis or by mobilization of stored material. Mature leaves are considered
sources because they tend to be associated with production and export of assimilates. All nongreen tissues and those not containing sufficient chlorophyll to meet their own food requirement, are sinks for the import of assimilates. These include apical and lateral meristems, all root tissues, storage organs such as fruits, seeds, tubers, etc. (Crafts and Crisp 1971). Sinks are defined as places removing sugars from the translocation system by consuming them (Hartt et al. 1964). Warren-Wilson (1972) used three criteria for defining sources and sinks, namely,

1. Transport: Sources are regions that export assimilates, while sinks import them.

2. Morphology: Mature leaves tend to be associated with production and export of assimilate, whereas other parts such as roots, meristems, fruits, and storage organs tend to be associated with import and utilization of assimilate. The terms source and sink are applied, therefore, to particular parts of the plant.

3. Metabolism: Sources produce assimilate by photosynthesis, while sinks utilize assimilate in respiration and growth.

Very often, terms such as source strength or sink strength are used to describe the degree of source or sink effectiveness. Warren-Wilson (1972) proposed that:

$$\text{Source strength} = \text{Source size} \times \text{Source activity}$$

or

$$\text{(Rate of assimilation) per plant} = (\text{Leaf area}) \times (\text{Rate of assimilation} \text{ per plant}) \text{ per unit leaf area}$$

while,
Sink strength = Sink size x Sink activity

or (Absolute growth rate) = (Dry weight) x (Relative growth rate)

It should be clear from such a description that one could cut down the sink strength by reducing the sink size with the assumption that the sink activity will remain the same. Strength refers to the absolute rate of change in weight of a substance for a plant part, and size is measured in terms of weight, area, or other characteristic.

Leaf area provides a generally accepted measure of the size of the source because photosynthesis depends on surface area. A method for measuring sink activity is not well developed (Warren-Wilson 1972).

Despite the lack of information concerning sink activity, use of radioactive carbon dioxide has given some information regarding sink strength. Hale and Weaver (1962), using vines of Vitis vinifera, demonstrated that fruit clusters are strong sinks when they are rapidly increasing in size. Flower clusters, on the other hand, are weak sinks because they have only a small influence on the movement of photosynthate.

Hansen (1967) found 90 per cent of the radioactivity taken up by the leaves of apples can be transferred to a fruit close by. He concluded that apple fruits act as strong sinks. The roots also are strong sinks in the plant. However, since the subject of this study is concerned especially with this sink, I have devoted a separate section for discussion of roots as major sinks.

In any system where competition takes place between parts for substances from a common and limited supply, the factor determining the
proportion of available solute reaching any part, apart from nearness of location, is the potential for consumption (Hill 1963). In other words, the strength of a sink (potential for consumption) will influence how much of the solutes will be available to other parts or other sinks in the plant. The distribution of assimilates appeared to be related not only to sink strength but also to proximity to the source and canalization effect imposed by the vascular system (Patrick 1972).

Sink regions are incapable of sustaining their own carbon requirements. Therefore, they exert a demand upon the translocation flow from the mature leaves (Webb and Gorham 1964). But the picture of uptake of assimilates by living tissues is still largely obscure. Kursanov (1963) suggested that the process of unloading and accumulation of sugars in sinks proceeds with the help of various mechanisms of a metabolic nature; and it is not determined by direct consumption of assimilate, but is accomplished by means of forces lying outside the sink and oriented toward a stimulus spreading from the growing zone. The nature of such a stimulus may be heteroauxin or other stimulants of biological origin.

In summary, it is generally agreed that source-sink relations regulate the rates of net transfer in the translocation system. The net transfer of sugars is from regions of production (source) to regions of consumption (sinks), but it is still not clear if this is a property of the flow in the phloem or of the arrangement for loading and unloading assimilates from the translocation stream.
Direction of Translocation

One of the few relatively clear aspects in the translocation process is the direction of movement. It is generally agreed that translocation proceeds from regions of production, "sources," to regions of consumption, "sinks." Accumulation of labeled translocates in the growing points was demonstrated by Aronoff (1955) and Barrier and Loomis (1957).

Translocation takes place along well marked gradients of sugar concentration (Mason and Maskell 1928). Using bean plants and radioactive carbon dioxide, Biddulph and Cory (1965) found that direction of assimilate movement appeared to be directly related to an intensity factor represented by concentration differences at source and sink, and inversely to a distance factor between the two, in other words to a gradient. They concluded that since each of a number of mature leaves serves as a source with the major sinks at opposite ends of the plant, the direction of the metabolites flowing between each is determined by a complex net gradient. Therefore, the lowermost leaves supply largely bulk sucrose export to the roots, while the uppermost export to the apex.

Thaine, Ovenden, and Turner (1959) supplied radioactive carbon dioxide to a limited area of a mature illuminated leaf of soybean. They found that direction of movement depends on the age and position of the leaf on the stem. They concluded that radioactive compounds move along a concentration gradient of the whole assimilate, labeled and unlabeled. They also noted that for the lowermost leaves, the major sink for
assimilates is the roots. For uppermost leaves it is the apex and young expanding leaves.

From the above discussion, it should be clear the direction of movement of assimilates could be upward or downward according to the relative position of sources to the sinks. This direction can be reversed if other sources or sinks are developed in the translocation system. For example, Hale and Weaver (1962) studied the effect of the developmental stage on direction of photosynthate translocation. They used radioactive carbon dioxide and by means of autoradiography followed the movement of C-14 photosynthate along vines of Vitis vinifera. They noticed that a young developing leaf at the shoot tip was at first parasitic on the rest of the vine and imported assimilates. With development, acropetal movement from the new exporting leaf lasted for one or two days, and with the development of one additional exporting leaf the direction of translocation from the first leaf became bi-directional. Bidirectional translocation lasted for only a short time before the direction of translocation was completely reversed and became strictly basipetal. The direction became bidirectional again after flowering and continued this way throughout ripening of the fruits.

The direction of translocation seems to be maintained by the unloading of assimilates from the phloem at the sinks regions. This will determine the concentration gradient (Nakata and Leopold 1967). It is interesting, though, that downward translocation was stimulated by ATP while upward translocation was not affected by ATP (Shiroya 1968).

In summary, direction of translocation is determined primarily by the relative position of sources and sinks of metabolites. This
direction follows a decreasing concentration gradient of assimilates, mainly sucrose. The unloading step for sugars at the sink region seems to regulate the direction through maintenance of a sugar gradient along the path.

**Distribution Pattern of Translocation**

The term distribution pattern can be roughly defined as the indicator of the proportion of total assimilates found in each part of the plant. Distribution pattern of assimilates, therefore, is a direct function of direction and rate of assimilate movement out of the source leaves. Subsequently, factors that affect the outflow from the source and those that affect the sink strength will determine primarily the distribution pattern (Wardlaw 1968).

It is a widely accepted point of view, that outflow of assimilates from a leaf is a function of its stage of growth. A young leaf forms part of the major apical sink for upward moving assimilates and for its own photosynthate products. It begins to function as an exporting organ after it reaches a certain stage of development (Thain et al. 1959, Shiroya et al. 1961, Thrower 1962, Webb and Gorham 1964, Köcher and Leonard 1971). Biochemical or physiological conditions of the source leaf will determine its effectiveness as a source (Shiroya 1968). The pattern of distribution is reflected primarily by the physiological activity of the leaf source (Patrick 1972).

Once a leaf is fully expanded, it is no longer capable of importing assimilates from other sources (Jones et al. 1959, Wardlaw 1968). However, mature fully expanded leaves were found to retain some
of their own photosynthate. This fraction will not be translocatable (Thaine et al. 1959), and seems to be used to satisfy the needs of exporting leaves.

The pattern of distribution will be determined, in part, by the ability of sinks to deplete the supply which will favor movement toward the growing organs (Wardlaw 1968). Active vein unloading appears to be a feasible mechanism for controlling distribution to regions upon demand (Geiger 1966).

Vascular interconnections significantly influence the distribution of photosynthates. Kursanov (1963) reported that in the source blade, the transport of assimilates in any position is oriented toward the closest conducting cells of the phloem. Distribution of radioactivity from a single leaf source was found to follow a well-defined pattern determined by the vascular interconnections (Jones et al. 1959, Shiroya et al. 1961, Patrick 1972, Larson 1972, Larson and Dickson 1973). Therefore, the position on the plant of both importing sites and exporting leaves will affect the distribution pattern. Presence of importing sites, such as immature fruits and meristems, and the position of these sites with respect to the supply leaves, are major determinants affecting the translocation pattern (Swanson 1959). Growth substances also were found to stimulate translocation rate and pattern (Hew, Nelson, and Krotkov 1967).

It was reported in a previous section that the apex is the major sink for the uppermost leaves, while it is the roots for the lowermost leaves. Accordingly, the intermediate leaves deliver to these two end sinks proportions consistent with their proximity to each end. If the
amount of assimilates moving upward from a single leaf is designated by
A, and the amount moving downward from the same leaf is designated by
B, then B/A will be defined as the distribution ratio (Thrower 1962).
This distribution ratio differs for each leaf, increasing as the source
leaf is nearer to the roots.

Complete distribution of assimilates takes place in a relatively
long time. It was found that after exposure of a source leaf for 10
minutes to radioactive carbon dioxide, complete distribution of C-14 was
attained after 6 hours from the exposure time (Jones et al. 1959, Clauss
et al, 1964). However, during the first two hours from the exposure
time, most of the exported C-14 was found in the ethanol soluble
fraction (i.e., translocatable forms).

To summarize, the distribution pattern of assimilates primarily
is ruled by the law of supply and demand. The supply of assimilates is
controlled by a source leaf through its photosynthetic capacity and
loading process. These in turn are regulated by the physiological
of development. On the other hand, the demand is manifested by the
ability of growing tissue to deplete the supply of sugars and therefore
maintain a gradient. Proximity of suppliers to the demand sites, as
well as presence of vascular interconnections between these sites, will
determine the final distribution pattern of the translocates.

Environmental Factors and Translocation

Since most of the internal factors affecting translocation and
distribution of photosynthate were covered in the previous sections, the
major emphasis in this part will be devoted to some of the external
factors that seem to affect the translocation process in one way or another. Temperature, light, concentration of carbon dioxide, and water status are some of these external factors.

At this point, it may be helpful to look at the translocation complex as one system composed of three subsystems. These are source, sink, and pathway. The external factors, as will be clear from the following discussion, may affect the translocation process directly or indirectly by their influences on one or more subsystems of the translocation complex.

Temperature

In 1952, Vernon and Aronoff found that chilling sections of soybean stems to 0°C resulted in drastic reduction in the rate of translocation. Similar results were obtained by Thrower (1965). He demonstrated that chilling a short length of petiole of soybean to 1°C prevented translocation of labelled assimilates past the chilled section. Hull's (1952) results showed that chilling temperatures of 1-3°C did not reduce translocation in tomatoes and sugar beets. In regard to the discrepancy of the results concerning the effect of low temperature on translocation at the path site, Geiger (1969) reviewed the subject and attributed any reduction of translocation by cooling the path to physical damage to the sieve tubes. Later, Geiger and Sovonick (1970) provided evidence that inhibition of mass transfer during petiolar cooling is a result of decreased translocation velocity.

'Temperature seems to have a significant role on translocation at the regions of source and sink. Barrier and Loomis (1957) reported
the $Q_{10}$ for translocation on the order of 2. They concluded that, since a $Q_{10}$ of 2 resembled the coefficient for enzymatically controlled processes, the effect of temperature lies in the step of vein loading. Geiger (1966) demonstrated the effect of cooling the sink region on translocation. He observed four phases of changes; a temporary decline, a period of translocation at the pretreatment site, a period of decline, and a new steady rate at 35-45 per cent of the original rate. Geiger explained these findings through the effect of temperature on the active unloading process, and the cooling effect was mainly due to inhibition of this metabolic process.

Support for these views came from the work of Coulson et al. (1972). Temperature effect on translocation was at either loading or unloading sites and not on the translocation path. Swanson (1959) reported an optimum temperature range of 20 to 30 C for translocation in Phaseolus vulgaris. The rate of translocation diminished at temperatures above and below this range. High temperatures may reduce translocation rate because they induce formation of callose on the sieve plates (McNairn 1972).

Light

Light affects the translocation process at least in two different ways. First, since it directly affects photosynthesis, the formation of translocates in the source leaves depends on light. Second, the process of vein loading requires energy. There is some evidence that ATP formed in non-cyclic photosynthetic photophosphorylation is used for the vein loading process (Plaut and Reinhold 1969). Translocation from a source
leaf in soybeans was found to be less under low light intensities than at higher light intensities (Thrower 1962). Following darkening of the source leaf of sugarbeets, Geiger and Batey (1967) noticed a rapid decline in translocation from this source. The direct effect of light on photosynthesis and as an indirect source of energy for vein loading seems to explain the action of light on translocation.

Carbon Dioxide Concentration

Varying the concentration of carbon dioxide in the atmosphere was found to have a direct effect on rate of photosynthate assimilation. Using bean plants, Mortimer (1959) demonstrated that rate of assimilation immediately increased approximately proportional to the concentration of surrounding carbon dioxide. Despite such increases in the rate of assimilation, Clauss et al. (1959) found carbon dioxide concentration has no direct effect on translocation in soybeans.

Later in 1961, Nelson et al. showed that the increase in the amount of C-14 assimilated was induced by increasing the total carbon dioxide concentration in the atmosphere. However, the amount of C-14 translocated was not significantly correlated with the increase. The limitation in translocation in such investigations is clearly not the availability of photosynthates but probably the loading capacity of the system and how much energy rich intermediates are present to energize the vein loading step. Recently, Christy and Swanson (1973) found that under steady state conditions the velocity of translocation appeared dependent on both the source leaf sucrose concentration and photosynthetic rate.
Water Status

Plaut and Reinhold (1965) studied the effect of water stress on C-14 sucrose transport in bean plants. They found C-14 which moved out of the leaf was much reduced by water stress. The slope of the distribution profile was found to be much steeper in stressed than in control plants. The effect of water stress on translocation was also investigated by Wardlaw (1969). He found the transfer of assimilates from the photosynthetic tissues into the conducting tissue was delayed in stressed leaves, but the velocity of the assimilate translocation through the conducting tissue was only slightly affected. Wardlaw suggested that effects of water stress on translocation arise indirectly from effects on growth. Reduced translocation in plants under water stress is the result of a reduction in growth rate (Wardlaw 1968).

Clor, Crafts, and Yamaguchi (1962, 1963), using seedlings of cotton, found that high relative humidity enhanced greatly movement of phloem transport and induced transport via the xylem.

Translocation of assimilates is also affected by many other factors such as growth substances (Hew et al. 1967) and level of nutrients in the medium (Pristupa and Kursanov 1957, Rangnekar 1973).

Roots: A Major Sink for Photosynthates

Roots of plants are heterotrophic organs. They lack chlorophyll and thus carry on no photosynthesis as do other plant organs. In this respect, roots differ from other sinks on the plant such as expanding leaves or developing fruits, because the latter contain chlorophyll and partially satisfy some of their carbon requirement through photosynthesis
in site. Roots, therefore, depend completely on shoots for supplying them with photosynthate.

Since the strength of a sink is a function of its size and activity, roots apparently are very strong sinks. Vernon and Aronoff (1952) studied translocation of C-14 in soybeans. From the second foliar node, radioactive products moved both acropetally and basipetally with the predominance of activity moving basipetally.

Nelson and Gorham (1959) introduced different labelled amino acids through the cut petiole of one primary leaf of soybeans. Translocation of each amino acid was found to be mainly toward the roots. Very little was translocated upward. Roots seem to act as strong sinks in a definite period in the life of the plant. Kursanov (1957) found up to 50 per cent of the assimilated material labelled with C-14 was directed from the leaves to the roots. However, in mature plants the flow of assimilate products into roots is only 1-5 per cent.

Shiroya et al. (1961) studied the distribution of the translocated sucrose. They found the bulk of translocated C-14 was recovered from the roots. Major downward movement, toward the roots was also demonstrated by Thrower (1962). He examined export of C-14 assimilates from different leaves of soybeans and found that in all cases movement down to the roots was greater than movement up to the apex and developing leaves. However, it is more marked in lower leaves than upper leaves. Starck (1963) found 59 per cent of C-14 translocated in bean plants was recovered in the root. Starck (1966) showed the share of roots in total translocate was high in bean plants, about 1/3 of C-14 substances exported from the blade. In a comparison of
upward and downward translocation of C-14 from a single leaf of the
sunflower, Shiroya (1968) found in young plants 35 per cent of the total
C-14 exported from the leaf was translocated upward and 65 per cent
downward. In the case of old plants only 5 per cent of total export
was upward and 95 per cent downward.

Strength of the root as a sink seems to be affected by its
degree of development. Nelson (1962) found in pine seedlings, plants
with well developed roots fixed four times more C-14 during photo­
synthesis and translocated 40 times more C-14 than plants with poorly
developed roots. Movement toward the roots is a fast process. Webb
and Gorham (1964) detected C-14 in the roots ten minutes after radio­
active carbon dioxide was assimilated by the primary leaf of the
straight-necked squash.

Although there is general agreement that roots are a major
importer for photosynthate produced by shoots, it is not clear however
if roots retain all this import or transport part of it back. Pristupa
and Kursanov (1957) using pumpkin plants showed that 18-48 per cent of
the total C-14 assimilate appeared in the root system. In view of this
significant accumulation of assimilate in roots, Pristupa and Kursanov
suggested that roots represent a large system for the transformation
process. The assimilated C-14 reaching the roots is converted into
substances of an ionic nature. This transformation process depends on
the external condition, such as availability of nutrient in the medium.
Therefore, it is closely related to the absorbing activity of the roots.
They showed that 60 per cent of the translocated C-14 to the roots was
retained in the roots. This fraction is utilized for their own growth.
The other 40 per cent of radioactive assimilate after transformation was transported back to the aerial organs. Unfortunately, there are no further evidences to support or refute such cycling of assimilate in the roots.

The role of roots in translocation of assimilates is not clear yet, since some workers such as Nelson and Gorham (1957b) and Starck (1964) suggested that roots have strong influences which favor translocation downward, but others such as Hartt et al. (1964) and Winter and Mortimer (1967) showed that roots do not exert a controlling influence on translocation but can contribute to its efficiency.

In summary, roots of plants, apparently, are strong sinks for carbohydrates. They use most of their imports in their own growth.

**Consequences of Alteration of Sink Size on Translocation**

Translocation of assimilates is a process, as shown from the previous sections, that proceeds from the source to the sink. The rate of translocation from a source to a specific sink is proportional to the relative strength and proximity of this sink with respect to other sinks on the plant. Competition for assimilates may arise among the different sinks if the supply by the source is limited. It was also shown that roots of many plants, including bean plants, are strong sinks for assimilates.

Thus, it seems appropriate to ask this question: What are the consequences of manipulation or elimination of part of or a whole sink, specifically the roots, on the translocation process as well as on the growth and development of the whole plant? In answering this question,
much will depend on the following two aspects. First, effect of manipulation of sink size on the translocation process with reference to rate and velocity of translocation and to total export from the source. Second, effect of manipulation of sink size on growth and development of plants in relation to growth rate and capacity for assimilation.

Effects of Manipulating Sink Size on the Translocation Process

Thrower (1965) compared translocation profiles in upper and lower stems from a source leaf in soybeans. He demonstrated that the main front of activity moved downward at a greater velocity than the upward movement. Thrower concluded that movements with different velocities may be associated with the size of the sink to which they are moving, and that the sink size is more important than a continuous photosynthetic supply of carbohydrates in determining the characteristics of movement to a sink under normal conditions. In agreement with such findings Starck (1966) reported that the pattern of C-14 distribution depends on the location of suitable acceptors and their mobilizing power. This power seems to depend mainly on the rate of growth of the given organ. That the presence of sinks in the plant affect the rate of translocation was also demonstrated by Hansen (1967).

Nelson and Gorham (1959) introduced 10 labelled amino acids through the cut petiole of one primary leaf in soybeans. They found that both excision of the roots and chilling the roots decreased the velocity of downward translocation of aspartic acid. Removal of the roots increased the proportion of aspartic acid that was moved upward.
Such an effect of sink size on the velocity of translocation was also reported by Starck (1963, 1964). Using sunflowers, lupine, and bean plants, Starck found that when the relative root size was such that the root system normally received a small share of the total translocated photosynthate, such as in sunflowers, removal of the roots did not reduce the total amount of C-14 translocated and only slightly changed the distribution of radioactivity in the whole plant. But when roots receive a greater percentage of translocated carbon, as in lupine and bean plants, derootment greatly changed the pattern of C-14 distribution. Starck suggested that root removal may influence the velocity of movement of organic substances in the conducting channel. Further investigations by Starck (1966) on the effect of root removal on translocation in beans revealed that excision of the roots seemed to affect the velocity (and/or rate) of translocation in the petiole and stem and in some cases decreased the total export from the blade. While Starck in 1963 was not clear whether removal of roots in beans has an effect on the total export from the source leaf, he later gave the impression that total export in some cases may be reduced (Starck 1964, 1966).

The velocity and pattern of assimilate translocation in wheat plants were investigated by Wardlaw (1965) in relation to grain number or sink size. Wardlaw found removing two-thirds of the grains from the head reduced the velocity of the assimilates moving up the stem and increased the downward velocity in the stem. However, the rate of movement out of the leaf was unaffected by the removal of grains.

Surprising results were obtained by Winter and Mortimer (1967) in attempting to establish a direct relationship between the metabolic
activity of the roots and the translocation of carbohydrates from the leaves. When a portion of sugarbeet roots was excised 30 minutes before assimilation of radioactive carbon dioxide by a leaf, the quantity of labelled sucrose exported during 30 minute intervals was reduced to about 25 per cent of normal, but the apparent velocity of translocation was not altered. Elimination of part of the sink in sugarbeets caused a decrease in the translocation rate (Geiger and Batey 1967).

It is apparent from the above discussion that manipulation of sink size does alter the translocation process. However, there is no agreement on its consequential effects. In some cases the effect was primarily on the source level, represented by total export, and in other cases rates of velocity of movement seemed to be mainly affected.

Effects of Manipulation of the Sink Size on Growth and Development of Plants

Attempts to cut down the sink size of roots in hopes that such treatment might result in making more assimilate available to other sinks have not been highly successful. Removal of up to 50 per cent of roots of barley and rye had no effect on the growth rate of the roots, but the growth rate of shoots decreased (Humphries 1958).

Buttrose and Mullins (1968) imposed different levels of root pruning on grapevine plants. They found, one week after the initial root pruning, that shoots of plants root-pruned up to 50 and 25 per cent of the control root volume were already shorter than control shoots. They interpreted the results as indicating that roots are a source of a growth substance required for normal shoot growth. Removal of 60 per cent of the roots had no detectable effect on either roots or shoot
growth of winter wheat (Andrews and Newman 1968), suggesting that some species have more roots than they need for maximum growth.

Because halving the roots did not halve the increment in growth, Maggs (1964) suggested that some kind of compensation must have occurred, and that roots normally operate well below the maximum of which they are capable. Using tomato plants, Cooper (1971) found that restriction of the root system by pruning had little effect on partitioning of the dry matter between the component parts of the plant, Cooper concluded that root pruning had little effect on the absolute growth rate of the component organs.

Results obtained from such experiments clearly demonstrate two facts. First, some species produce more roots than they need for maximum shoot growth under favorable conditions. Second, translocation of assimilates is a process, just like other biological processes, that proceeds under the control of check mechanisms. Although the nature of such mechanisms is not clear yet it may be some kind of feedback inhibition. Burt (1964, 1966) showed that removal or chilling of potato tubers resulted in increased dry matter accumulation in the leaves, and the net assimilation rate was considerably reduced. Burt attributed such reduction in net assimilation rate to a decreased rate of photosynthesis.

Humphries (1963) reinvestigated the phenomenon of inhibition of assimilation by carbohydrate accumulation. He gave evidence that it is the rate of translocation of carbohydrate from the source to the sink and not accumulation of products that inhibits photosynthesis. That
greater translocation rates may cause greater rate of photosynthesis was also suggested by Hansen (1967).

The effect of sink size on photosynthesis was demonstrated by Humphries and Thorne (1964). They found that rate of apparent photosynthesis increased as roots grew, decreased when roots were removed, and increased again as roots regenerated. These results suggested that photosynthesis is controlled by sink size. Thorne and Evans (1964) also showed that greater use of photosynthate can increase rate of photosynthesis.

Neales and Incoll (1968) reviewed the hypothesis that leaf photosynthesis rate is controlled by the level of assimilate concentration in the leaf. They concluded that a satisfactory proof of this hypothesis must depend upon two levels of investigations: (1) the negative correlation between photosynthetic rate and assimilate level in the leaf, and (2) the biochemical mechanism involved. We have some information on the first level but none on the second level.

In summary, manipulation of sink size has some direct effect on the translocation process. It affects rate and/or the velocity of translocation. Its effect on total export from the source has not been clearly demonstrated, but apparently reducing sink size will decrease the total export from the source. Subsequently, accumulation of assimilates will take place in the source resulting in stimulation of some unknown feedback mechanism which finally reduces the rate of photosynthesis. Although such a model can readily explain some of the results obtained from very simple systems like Humphries and Thorne's (1964), it is by no means sufficient to explain the situation in larger
systems having many other sinks ready to take over any excess of assimilates.
OBJECTIVES OF THE PRESENT STUDY

From the above discussion it appears that translocation of photosynthates, mostly sucrose, from their sites of assimilation to the sites of utilization, is a process of great potential rate and a mechanism of long distance transport seems to be involved. Although the nature of this mechanism is not fully understood, translocation physiologists agree that the direction of photosynthetic translocation follows primarily the concept of source/sink relations, that photosynthates move from the source "expanded photosynthetic leaves" to the different sinks "all non-photosynthetic living tissues plus some photosynthetic ones too." However, rates of photosynthetic translocation to the different sinks such as roots, small expanding leaves, developing fruits, and growing points are dependent largely on the strength of these sinks.

Roots are considered as very strong sinks because they draw more than 50 per cent of the total translocated photosynthetic. Many studies showed the significance of roots as organs of absorption of water and nutrients, but effects of manipulating the strength of this sink on translocation of photosynthetic has not been studied in great depth. Since distribution of photosynthates is governed primarily by the law of supply and demand, cutting down the strength of the root sink is likely to alter this distribution and should have some direct or indirect effects on the dynamics of the translocation process itself.
Sink strength was shown to be a function of the product of sink size and sink activity. Therefore, removal of half of the root size by pruning, is likely to cut down the strength of this sink by one half followed by half the original demand for photosynthate. Manipulation of sink size is likely to result in either more photosynthate becoming available for other sinks, and this should be reflected in more growth for these sinks if their mobilization power is not limited and availability of photosynthate is the limiting factor; or, the sink size itself having some control on photosynthate assimilation and translocation with no net gain in photosynthate by other sinks. The purpose of this study was to test such hypotheses, that is, to determine effects of manipulation of root size on the growth of shoots and on the movement of photosynthate from specified sources to some known sinks.
MATERIALS AND METHODS

Plant Material and General Method Used

Red kidney bean (*Phaseolus vulgaris* L.) plants were used for this study because they have been used very often in relation to translocation studies (Biddulph et al. 1958; Biddulph and Cory 1965; Humphries 1963; Köcher and Leonard 1971; Leonard and King 1968; Mortimer 1959; Nakata and Leopold 1967; Plaut and Reinhold 1969; Starck 1963, 1964, 1966; Weidner 1964) and because of their relatively short life cycle.

The method used for germination and growing the plants in all the experiments was as follows. Seeds were soaked for 5 minutes in one per cent solution of Clorox, then rinsed in running water for 15 minutes, and finally washed with distilled water. Ten seeds were placed on sterile brown paper towels soaked with 80 ml of nutrient solution (O'Leary and Prisco 1970) and covered with plastic wrap, rolled up, and placed vertically in plastic beakers. The beakers were then placed in a dark, warm place, 28±2 C, for four days. The paper towels were opened and the healthy small seedlings were placed on new sterile brown paper towels soaked with fresh nutrient solution. Seedlings were then placed in the condition of the particular experiment (conditions of each experiment will be specified). Seven days from planting, seedlings were transferred to aerated nutrient solution in containers that will also be specified for each experiment. Changes of the nutrient solution were
made every week in the early stages, then every 3 to 4 days in the advanced stages of growth.

Roots were pruned to the required size, visually by eye, using scissors. In long term experiments, root size was maintained at the desired level by weekly check pruning.

Unless otherwise specified, all chemicals used in this study were Analytical Reagent Grade.

**Measurements of Relative Water Content**

Roots of plants are known to function primarily in absorption of water and nutrients. To determine the lower limit of root size that would not alter the water status of the plant, as measured by the relative water content, 48 selected plants were transferred to aerated nutrient solutions. Each plant was placed in a one liter glass beaker wrapped with aluminum foil and covered with a six inch square one fourth inch plywood top with a slot to facilitate plant removal, a wooden support dowel, and a butyl rubber flap over the slot. The beakers were then placed in an Environmental Growth Chamber, Model M-2, programmed for a 14 hour light, 10 hour dark cycle. The photoperiod was arranged for only one-third light intensity to be at the first and last hours while full light intensity was arranged for the middle 12 hours. Full light intensity at the level of the plants was about 3000 ft-c. as measured with a Weston Illumination Meter, Model 756. The sources of light in the chamber were cool white fluorescent, incandescent, and tungsten tubes. The temperature of the light period was 27±2 C and in
the dark period it was 22±2 C. The relative humidity was 50±5 per cent during the light period and 75±5 per cent during the dark period.

When the plants were 3 weeks old, the primary leaves, first trifoliate, and second trifoliate leaves were fully expanded. At this stage of development different levels of root pruning were imposed. Levels of root pruning were R100, R75, R50, and R25 (indicating percentage of the root remaining after pruning, relative to plants with complete roots R100). Twelve plants were sampled every 24 hours for 96 hours. Plants were distributed in the chamber in a complete randomized design with three replications.

The method used to measure the relative water content (RWC) was described by Weatherley (1950) and re-examined by Barrs and Weatherley (1962). On the day of sampling 12 plants were divided into primary leaves, first trifoliate, and second trifoliate leaves. The method of sampling was to punch discs of leaf tissue with a cork borer. The borer was approximately 0.75 cm in diameter. The main veins of leaves were avoided and the position chosen for punching was restricted to areas between the main and lateral veins. Forty discs were obtained in this way from each leaf (the two primary leaves were considered as one leaf). The fresh weight of discs was measured to the nearest milligram. Leaf discs were then floated in petri dishes filled with cold distilled water. The floated discs were arranged with forceps so the upper surface of the discs was on top. Petri dishes were then placed in a cold, 2-3 C, dark chamber. After 24 hours, disc surfaces were dried, gently and quickly, with soft paper tissue. Full turgid weight of discs was then measured. To obtain the dry weight, leaf discs were
placed in dry petri dishes, covered on the bottom with aluminum foil. Samples were then placed in a drying oven at a temperature of 70±2°C for 24 hours. The dried leaf discs were then weighed to the nearest milligram.

In this way, fresh weight, turgid weight, and dry weight of the samples were determined. The RWC was then obtained according to the following equation:

\[
\text{RWC} = \frac{\text{Fresh weight of discs} - \text{dry weight}}{\text{Turgid weight of discs} - \text{dry weight}} \times 100.
\]

Data obtained were subjected to variance analysis.

Partitioning of Dry Matter and Some Growth Characteristics

Plants for this experiment were grown under the same conditions as the experiment described in the relative water content experiment. Forty-eight plants were arranged in a complete random design to give three levels of root sizes (R100, R75, and R50) and four weekly harvesting intervals, with four replications. Root pruning was done when the plants were three weeks old from planting. The removed roots were wrapped in aluminum foil and placed in an oven at 70±2°C for 48 hours. Their dry weight was then determined. Three days after the first root pruning 12 plants were removed. The harvested plants were divided into leaves, stems + petioles, roots, and pods (if any). The leaf area for each plant was measured by tracing the outline of the leaves on a paper having a uniform weight distribution with area. The leaf shapes were cut from the paper and weighed. The leaf area was then calculated from the weight to area relationship of the paper,
established by weighing pieces of paper of known area. The different
plant parts were placed in paper bags and dried in an oven at 70±2 C
until the dry weights remained fairly constant. Dry weights were then
determined. This procedure was followed for the rest of the plant
harvesting in the succeeding intervals.

Root size for all plants was maintained at the required level
by weekly check pruning. This was done 3 days before each harvest. In
this way, the interval period between harvests was one week, and between
root pruning was also one week, while between root pruning and harvest
was 3 days.

The roots removed at each pruning were dried, and dry weights
were then added to determine the total accumulated dry weight of roots
for every plant. From the data obtained, net assimilation rate and
leaf dry weight/leaf area ratio were found.

**Carbon Dioxide Concentration**

Carbon dioxide concentration in plant microclimate was found to
affect rate of formation of photosynthates. Higher concentrations of
carbon dioxide increased formation of photosynthate (Mortimer 1959).
The purpose of this experiment was to determine if conditions stimu-
lating formation of photosynthate would have any interaction with root
size on growth and yield of plants. In other words, if the level of
photosynthate in the source leaves increased, what would be the effects
of manipulation of root size on growth and yield of plants? To test for
this, three identical growth chambers, ISCO Model E-3, were programmed
to 12 hours light followed by 12 hours dark. The photoperiod was
divided so that one-third of light intensity came in the first and last half hours, while full light intensity for the middle 11 hours. Full light intensity at the level of plants was about 4000 ft.c. as monitored by a Weston Illumination Meter Model 756. The light source in each chamber was composed of 3200 watts of Sylvania Metalarc lamps (metal halide vapor) and 800 watts of incandescent light. The temperature of the light period was 24 C and that of the dark period was 19 C. Relative humidity was controlled by an electronic control system and was maintained to 70±5 per cent all the time. The levels of carbon dioxide concentrations chosen were 400, 800, and 1200 ppm. The three growth chambers were connected to a Beckman infrared gas analyzer, which monitored continuously the concentrations of carbon dioxide for each chamber alternatively. The gas analyzer was attached to three solenoid valves which each injected, upon demand, specific amounts of carbon dioxide through flow meters, into the corresponding chamber. The solenoid valves were connected to a supply of carbon dioxide (Fig. 1). To reduce leaking of carbon dioxide from the chambers, heavy plexiglass covers were installed on the fronts of each chamber and tied to the edges with steel screws (Fig. 2). In this way the concentration of carbon dioxide was maintained automatically at the required level for each chamber during the whole term of the experiment.

Twenty plants were transferred to aerated nutrient solution in each chamber. Each plant was placed in a one liter plastic beaker. The beakers were fixed in place and were connected to each other and to the outside by means of plastic tubing to facilitate changing of the
Fig. 1. Photograph of the system used to control the concentrations of carbon dioxide in the three different growth chambers.

Fig. 2. Photograph of the front of a growth chamber with the plexiglass cover in place -- Note the plastic tubing at the bottom for changing the nutrient solution.
nutrient solution without removing the chamber front (Fig. 2). The beakers were covered with opaque plastic tops.

When the plants were 12 days old from planting, half the plants in each chamber were pruned to R50, the other half left with complete roots R100. Roots were then maintained at this level by continuous check pruning every 4 days. The removed roots were dried and weighed after every root pruning.

At flowering, 10 plants were harvested from each chamber, 5 plants of R100 and 5 of R50. The removed plants were divided into leaves, stem + petiole, and roots. The leaf area was measured in a similar way as described in the previous experiment. The dry weight was determined for the different plant parts.

When pods of the remaining plants were filled, plants were harvested. The dry weight of roots and of pods was determined as well as the number of pods on each plant.

High Relative Humidity and Night Temperature Effects

Temperature was shown to have some effect on translocation of photosynthate in plants. Translocation is a temperature dependent process at least in loading and unloading of photosynthate into and from the translocation channel (Coulson et al. 1972).

While realizing that translocation of photosynthate occurs mainly during the day, the excess of photosynthate accumulated in leaves may also be translocated during the night. Optimum range of temperature for translocation in beans was found to be 20 to 30°C (Swanson 1959). Low night temperature is likely also to cut down the respiration rate
and results in more assimilate available for growth. The interaction
between root size and night temperatures on yield of bean plants grown
under high relative humidity was investigated.

The plants were grown in three air inflated greenhouses covered
with 12 mil polyethylene plastic (GER Pak 601). Day temperature was
29±2 C in all houses and for the night, temperatures were 29, 24, and
19±2 C for the three different greenhouses. Natural light intensity
under the plastic cover ranged from 4000-8000 ft-c. during the day. The
relative humidity was 85±5 per cent during the day and it was always 100
per cent during the night.

One hundred plants were placed into each house. The plants were
transferred to aerated nutrient solution in five liter plastic buckets.
The buckets were fixed in place and covered with wooden tops. The
marginal 40 plants were not under experimentation, and were removed
before harvesting. The 60 plants left were subjected to different root
treatments as follows:

1. 12 plants, R100.
2. 12 plants, R75, root pruning was started when plants were two
   weeks old.
3. 12 plants, R50, root pruning was started when plants were two
   weeks old.
4. 12 plants, R75, root pruning was started when plants were at
   flowering.
5. 12 plants, R50, root pruning was started when plants were at
   flowering.

Plants of each treatment were distributed in a complete randomized
design with 12 replications. Root sizes were then maintained at the required level by check pruning every week. The removed roots were dried and weighed. When plants reached maturity, all plants were harvested, and dry weight of pod and roots was determined.

**Ethanol Soluble Sugars**

Since translocatable photosynthate, mostly sucrose, moves from the source leaves to the different sinks, removal of part of the roots is likely to change the level of sugars in other parts of the plant. To test this, 24 plants were grown in aerated nutrient solution in Environmental Growth Chamber, Model M-13, programmed for 14 hours light followed by 10 hours dark. The light intensity at the level of plants was 2500 ft. c, as measured by Weston Illumination Meter. The light period was divided so that one-third of the light was used for the first and last hours, but full light was used for the middle 12 hours. The temperature of the light period was 27±2 C and for the dark period was 19±2 C. The relative humidity was 50±5 per cent for the light period and it was 75±5 per cent for the dark period. The plants were grown in containers the same as described in the relative water content experiment.

When the plants were three weeks old from planting, the primary and the first three foliar leaves were fully expanded, the fourth trifoliate was still small. At this stage, all plants were simplified by defoliation to one source leaf (middle leaflet of the second trifoliate) and two opposite sinks, these are the expanding fourth trifoliate upward and the roots downward (Fig. 3). Purposes of defoliation were to
Fig. 3. Simplified plant used in studying the content of soluble sugars -- (A) Photograph of the simplified plant used in this experiment (25 cm² grid background). (B) Diagram of this plant illustrating the locations of the parts used for the measurement.
eliminate the complicating effects of other exporting leaves (Hale and Weaver 1962) and to provide low carbohydrate background. Therefore, detection of the effect of the treatment on sugar content was feasible. Defoliation of other leaves except the source was shown to have no effect on translocation capacity from a single leaf (Hartt et al., 1964).

After 24 hours from defoliation, two levels of root size, R100 and R50, were imposed. The plants were then transferred to aerated distilled water, because preliminary experiments showed that such simplified plants are susceptible to mineral toxicity upon pruning of roots if they are placed into nutrient solution. However, depletion of nutrients from the medium was shown to have no effect on the translocation of assimilates (Winter and Mortimer 1967). After 24 hours from root pruning, all plants were removed, and the plants were divided into the source leaf, petiole, upper stem, and lower stem (Fig. 3b). The method of sampling the leaf was to punch discs of leaf tissue with a cork borer. The borer was 0.5 cm in diameter. Punching was restricted to areas between the main lateral veins. In this way, 40 discs were obtained weighing about 0.15 gram. The method of sampling petiole and stem was to cut 1 cm segments with a double bladed knife. Three segments were obtained from the petiole and another three segments from the upper stem. For the lower stem, two samples were taken; two segments from the internode just below the source leaf and three segments far down just above the transition zone (Fig. 3b). The distance between the two samples of lower stem was measured. Using a Mettler balance, the fresh weight of the samples was determined to the nearest milligram. The samples were transferred then to 5 ml test tubes
and they were immediately frozen by liquid nitrogen. The test tubes were then sealed with parafilm and stored in a deep freeze at -20 C until extraction.

The translocation sugars were extracted with 80 per cent ethanol because this procedure has been used extensively in many translocation studies; the method is described by McCready et al. (1950). The frozen samples were sliced into small pieces with a sharp razor blade. Each sample was then transferred to Whatman No. 1, one layer cellulose extraction thimble 55x10 mm. The extraction of soluble sugars was then carried out in a Soxhlet with 25 ml of 80 per cent ethanol. Soxhlet apparatuses were placed in an electrically heated sand box. The temperature of the sand was 90±5 C. Under such conditions the alcohol was boiling gently during the whole period of extraction, 12 hours. Preliminary studies showed that this procedure is sufficient to extract almost all the alcohol-soluble sugars.

The alcohol extract was then filtered on Whatman No. 1 filter paper and the filtrate was evaporated to dryness in rotating flash evaporators at 35 C (Fisher 1970a). Sugars were then taken in 10 ml of distilled water and transferred to a 20 ml centrifuge tube, 10 ml of methylene chloride was then added to the extract to separate the soluble pigments. Samples were centrifuged at 15,000 rpm for 10 minutes. The water phase on the top, which contained the sugars, was taken by disposable pipette into 25 ml volumetric flask and the final volume was brought to 25 ml with distilled water. The flasks were sealed with parafilm and stored in refrigerators for 24 hours.
Concentration of sugars in the extract was determined colorimetrically following, in principle, the method described by Yemm and Willis (1954). Five ml of chilled anthrone reagent (0.2 g anthrone + 47.5 ml sulfuric acid + 2.5 ml distilled water) were added to 2 ml of ice cold sugar extract. The mixture was then heated in boiling water for 10 minutes, then cooled in ice bath and the optical density was measured at 625 nm in Beckman Spectrophotometer Model 1098. Standard samples of known sugar concentration were run with the extract samples. In this way, the concentration of sugars in the extract was determined from the linear relationship between concentration of sugars and their optical densities. Corrections for dilution were made, and the concentration of sugars was expressed on μg per gram fresh weight or on μg per centimeter basis.

Translocation of Sucrose C-14

The 24 plants used in this experiment were grown in aerated nutrient solution in conditions similar to the one described for the ethanol soluble sugar experiment. All plants were simplified by defoliation to one source and two sinks as described before (Fig. 3a). Root treatment R100 and R50 was carried out 24 hours after defoliation and plants were then transferred to aerated distilled water. The source leaves were levelled horizontally on a meter stick fixed on two stands. Plants were left in this condition for 24 hours and during the time of introduction of labelled sucrose.

Uniform sucrose C-14 solution, specific gravity 200 μc ml⁻¹, was prepared and small amounts of Tween 20 and boric acid were added to the
sucrose solution to facilitate the entry of sucrose into the cells of non-wounded leaves (Clor et al. 1962, Plaut and Reinhold 1965, Leonard and King 1968). The method used for introduction of sucrose C-14 was described by Nelson and Gorham (1957a). It was shown (Plaut and Reinhold 1965) that the sucrose introduced in this manner moved in the sieve tubes in a similar way to synthesized sucrose. A ring, 1 cm in diameter and 0.5 cm thick, from tygon tubing was stuck with grease to the upper surface of each source leaf, at about 1 cm from the midrib. Using a micropipette, 0.1 ml of the sucrose C-14 solution was poured inside the well. In this way each plant received 20 μc. Four plants, 2 of R100 and 2 of R50, were harvested after 1/4, 1/2, 1, 2, 4, and 8 hours from the time of sucrose application.

At harvesting, the rings and the leaf tissue underneath it were removed by punching with a cork borer. The plants were then mounted on cardboard sheets and covered with plastic wrap. Mounted plants were frozen by liquid nitrogen and pressed in a plant press. The plants were then placed against a no-screen X-ray film (35.5 x 10.5 cm), enclosed in a film holder and stored in a deep freezer for 2 weeks. The resulting films were developed and the appropriate pictures were taken.

Translocation Velocity of Labelled Photosynthate

The 24 plants used in this experiment were grown in the same conditions described for the ethanol sugar experiment. Plants were maintained at one stem by continuous removal of lateral branches. All plants were defoliated after six weeks to one source and two opposite sinks. The source was the middle leaflet of the third trifoliate. The
two sinks were the 8-10 expanding pods and the roots. About 24 hours before labelling with radioactive carbon dioxide, roots of 12 plants were pruned to R50, the other 12 plants were left with complete root systems R100.

The method used in this experiment for labelling and sampling was developed from different methods (Wardlaw 1965, Plaut and Reinhold 1969, Fisher 1970a). Four plants, 2 (R50) + 2 (R100), were fed C-14 simultaneously by enclosing their source leaves in an assembled rectangular Plexiglass feeding chamber, 56 x 14 x 3 cm. The petioles were sealed into the chamber with modeling clay (Winter and Mortimer 1967, Fisher 1970a). The leaf chamber was included in a closed air circulated system (Fig. 4 and 5). Plastic tubing connected the parts of the system. The air was circulated at a rate of 1200 ml/min.

Radioactive carbon dioxide was generated hypodermically, by adding 10 ml of 17 per cent lactic acid to 90 mg of Ba\(^{14}\)CO\(_3\), specific activity 0.5 μc. Preliminary investigations showed that using this amount of barium carbonate was sufficient to bring the concentration of carbon dioxide to 500 ppm in the volume of the system.

The source leaves were exposed to \(^{14}\)CO\(_2\) for 10 minutes under water filtered light of 4000 ft.c. The light source was 2000 watts of photoflood lamps. The circulated air was then freed of CO\(_2\) by passing through 10 per cent solution of sodium hydroxide for 5 minutes (Pristupa and Kursanov 1957, Starck 1964). The top of the leaf chamber was then removed, and the plants were left for 15 and 30 minutes translocation periods, excluding the feeding time. Two plants were removed after 15 minutes and divided into upper and lower stems based on their position.
Fig. 4. Apparatus used in assimilation and labeling of the plants with C-14 -- (A) Photograph of the parts of the system. (B) The $^{14}\text{CO}_2$ generator and absorber.
Fig. 5. Schematic drawing of apparatus used for assimilation and labeling the plants with C-14.
relative to the source leaf. Stems were then frozen in liquid nitrogen and stored in a deep freezer. The other two plants were harvested after 30 minutes and were divided in a similar manner. In this way, all experimental plants were labelled and sampled.

At extraction, frozen stems were divided into 1 cm sections by a double bladed knife, each section was then sliced into small pieces and extracted for ethanol soluble photosynthate by 80 per cent alcohol. The method of extraction was the same as the one described in the previous experiment. The ethanol soluble extract was transferred quantitatively to 20 ml scintillation vials and evaporated to dryness in an air forced oven at 45 C. One ml of hydrogen peroxide, 30 per cent, was added to each sample and the samples dried again under the same condition. Fifteen ml of scintillation system (24 ml Toluene:1 ml Permaflour "Packard") was added to each vial. Radioactivity was then measured for each vial in a Packard-Tricarb Liquid Scintillation Spectrometer Model 2002.

**Distribution of Ethanol Soluble Photosynthate C-14 in Simplified Plants**

Details for growth conditions were the same as described in the ethanol soluble sugar experiment. The method used for labelling plants with C-14 was similar to the one described in translocation velocity of photosynthate; however in this experiment the translocation period was 90 minutes including feeding time. It was shown by others (Clauss et al, 1964) that during such periods most of the labelled photosynthates are still soluble in ethanol. The 12 experimental plants, 3 weeks old, were divided into source leaflet, petiole, upper stem including the
fourth expanding trifoliate, lower stem, and roots. Each part was frozen by liquid nitrogen and extracted for ethanol soluble photosynthate using the method described previously. Volume of the alcoholic extract was brought to 50 ml by 80 per cent ethanol, 10 ml of the extract was then placed in scintillation vials and the radioactivity was counted using the same procedure described in the past experiment. The 40 ml of the alcoholic extract left was evaporated to dryness in a rotary flash evaporator at 35 C, and the dried extract was then taken in 3 ml of 80 per cent ethanol.

Ethanol solution compounds were separated by descending paper chromatography on 21 x 57 cm sheets of Whatman No. 1 chromatography paper. Duplicate samples 0.1 ml each from the concentrated alcoholic extract were applied directly to the chromatography paper. The solvent used for separation was prepared by mixing N-Butanol, acetic acid, water in a ratio 4:1:5 by volume, the epiphase was then used for separation. Chromatograms were developed in the long direction for 36 hours. Sugars were identified by spraying the dry chromatograms with benzidine trichloroacetic acid and heated for two minutes in an oven at 100±5 C (Bacon and Edelman 1951). Spots of sucrose, glucose, and fructose were cut from the chromatogram, placed in scintillation vials, and the radioactivity in each spot was assayed using a liquid scintillation system the same as described previously.

In this way the total activity in each part of the plant was found as well as the percentage of activity in the main three sugars.
Distribution of Ethanol Soluble and Insoluble Labelled Photosynthate in Complete Plants

The eight plants used in this experiment were grown in aerated nutrient solution under the same conditions described in the experiment of ethanol soluble sugars. When the plants were 3 weeks old from planting, roots of four plants were pruned to R50, roots of the other four plants were left unpruned, R100. Twenty-four hours from the time of root pruning, the middle leaflet of the second trifoliate of every four plants were exposed simultaneously to radioactive carbon dioxide for 10 minutes. The method used for generation and labelling by C-14 was the same as described in the experiment of translocation velocity; however, in this experiment every four plants (2 of R50 and 2 of R100) were exposed to carbon dioxide of specific activity of 1 mc. Plants were then transferred back to the growth chamber for a translocation period of 6 hours. It was shown by others (Clauss et al, 1964) that distribution of C-14 in soybean was completed in 6 hours.

At harvest, all plants were divided into different leaves, different stem internodes, and roots (Fig. 6). Each part was then frozen in liquid nitrogen and stored in a deep freezer. The method used for extraction of the ethanol soluble photosynthate was the same as described in the experiment of ethanol soluble sugars. However, the extraction period was extended to 24 hours because of the relatively large amount of sample tissue. The alcohol extract was then brought to 50 ml by adding 80 per cent ethanol. Two subsamples from the alcohol extract, each 10 ml, were placed in scintillation vials, dried, and
Fig. 6. Diagram of the bean plant used in the study of the distribution pattern of C-14, giving the nomenclature of the various divisions.
radioactivity was determined using the same method described in the translocation velocity experiment.

Plant residue, after extraction by alcohol was dried, weighed, and ground into fine powder. Twenty mg of this powder was then homogenized with 2 ml of 80 per cent ethanol in a glass to glass homogenizer. One-half ml of the homogenate was pipetted on circles 2.3 cm in diameter of Whatman No. 3 filter paper. The circles were then dried, placed in scintillation vials and the radioactivity was determined (Keck 1973). In this way the radioactivity of the ethanol soluble and insoluble fractions was found for the different plant parts.

**Net Photosynthetic Rate**

In a closed system for analysis of gas exchange (Fig. 7 and 8), net photosynthetic rates were measured for 24 plants. The plants were grown under the same conditions described in the ethanol soluble sugar experiment. The plants were simplified to one source leaf and two sinks in the same way reported in that experiment. Roots of 12 plants were pruned to R50 while the other 12 plants were left with complete root systems, R100. All plants were then transferred to aerated distilled water.

The net carbon dioxide uptake was measured for four plants simultaneously by enclosing their source leaves in the leaf chamber. By means of Beckman infrared gas analyzer and a recorder the change in concentration of carbon dioxide during 30 mintues was found. The initial concentration of carbon dioxide in the system was adjusted to about 600 ppm. This was achieved by flushing the system with standard air,
Fig. 7. Apparatus used for measuring the net photosynthetic rate -- (A) View of the various parts of the system. (B) Close-up view of the placement and sealing of source leaves in the leaf chamber.
Fig. 8. Schematic drawing of apparatus used for measuring the net CO₂ uptake.
containing about 633 ppm carbon dioxide for a few minutes. Volume of air in all parts of the system was about 16 liters, and the rate of circulation was 1200 ml min\(^{-1}\). During the light period, the temperature of the circulating air was 28±2 °C. The leaf area of the source leaves was measured by the same method described in the partitioning of dry matter experiment. The net photosynthetic rate expressed as mg CO\(_2\) dm\(^{-2}\) hr\(^{-1}\) was calculated as follows:

\[
\text{Net Photosynthetic Rate} = \frac{\Delta \text{ ppm} \cdot 10^{-6} \times 44.000 \times \text{vol. system} \times P/760 \times T/300}{\text{leaf area (dm}^2\text{)} \times 22.4 \times T/60 \text{ min}}
\]

In this way the net rate of photosynthesis was compared between plants of pruned roots vs. plants with a complete root system every 24 hours for 72 hours.

**Kinetin Spray**

The 20 plants used in this experiment were grown under similar conditions described in the ethanol soluble sugars experiment. When plants were two weeks old, they were divided into two groups: Five plants from each group were root pruned to R50, and the root size was then maintained at this level by weekly check pruning throughout the experiment. After root pruning was started, one group was sprayed, until dripping wet, with a solution of 10 ppm Kinetin (Thrower 1964). Spraying with Kinetin was done daily until the plants were harvested. The plants were arranged for 2 x 2 factorial in complete randomized design.
When pods were fully filled, all plants were removed and separated into leaves, stems, and petioles and pods. Fresh and dry weights were then determined for these different plant parts.
RESULTS

Influence of Root Size on the Relative Water Content of the Plant Leaves

Details of the influence of root size on the relative water content (RWC) are presented in Table 1 and Fig. 9. The results showed that removal of up to 75 per cent of the roots did not alter the RWC of the three leaves investigated 24 hours from the time of root pruning. However, removal of more than 50 per cent of the roots reduced the RWC of the first and the second foliar leaves gradually, and the RWC became significantly lower than the controls after 96 hours. However, the RWC of those leaves recovered to the level of the controls within 24 hours.

Relative water content of the primary leaves was the least affected by root pruning. The RWC of the second foliar leaf was the most sensitive to pruning of the root. These results suggested that removal of up to 50 per cent of the root system did not alter significantly the water status of red kidney bean leaves grown under these conditions.

Influence of Root Size on Partitioning of Dry Matter and on Some Growth Characteristics

Removal of 25 or 50 per cent of red kidney bean roots did not significantly alter the growth or the distribution of the dry matter in the different parts of the plants, including the roots (Table 2). Leaf area per plant was not significantly reduced by removing part of the roots. The ratio of leaf dry weight/leaf area was slightly higher for
Table 1. Influence of root size on the relative water content of Red Kidney Bean leaves.

<table>
<thead>
<tr>
<th>Root treatment</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
<th>96 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P (a)</td>
<td>(L_1)</td>
<td>(L_2)</td>
<td></td>
</tr>
<tr>
<td>R100</td>
<td>84.2</td>
<td>85.1</td>
<td>80.5</td>
<td>84.0</td>
</tr>
<tr>
<td>R 75</td>
<td>85.5</td>
<td>81.9</td>
<td>80.4</td>
<td>88.8</td>
</tr>
<tr>
<td>R 50</td>
<td>84.0</td>
<td>84.9</td>
<td>81.8</td>
<td>84.5</td>
</tr>
<tr>
<td>R 25</td>
<td>86.9</td>
<td>83.3</td>
<td>81.3</td>
<td>86.5</td>
</tr>
</tbody>
</table>

\(a\): Primary leaves; \(L_1\): First foliar; \(L_2\): Second foliar.

*Indicates significant difference between root treatments at 0.05 level of probability.
Fig. 9. Effect of manipulation of root size on the RWC of the different leaves of the plant — (---*---), R100; (*—*—*), R75; (x—x—x), R50; and (o — o — o), R25.
Table 2. Influence of root size on the dry weight of the different plant parts.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>3rd Week</th>
<th>4th Week</th>
<th>5th Week</th>
<th>6th Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
<td>R 75</td>
<td>R 50</td>
<td>R100</td>
</tr>
<tr>
<td>Leaves</td>
<td>2.54</td>
<td>2.67</td>
<td>2.83</td>
<td>5.29</td>
</tr>
<tr>
<td>Stem + Petiole</td>
<td>1.26</td>
<td>1.25</td>
<td>1.33</td>
<td>2.84</td>
</tr>
<tr>
<td>Pods</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Root</td>
<td>0.80</td>
<td>0.80</td>
<td>0.86</td>
<td>1.42</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Statistical analysis of variance revealed no significant difference between the root treatments.
plants with part of the root removed than for plants with complete root systems (Table 3). The influence of root size on net assimilation rate is shown in Table 4. The data indicate that removal of part of the root system resulted in an initial reduction in the net assimilation rate during the first week. However, during the following weeks, the net assimilation rate was higher in the pruned plants than in plants with a complete root system.

Influence of Root Size on Growth of Red Kidney Bean Plants Grown Under Different Concentrations of Carbon Dioxide

The dry weight of the different plant parts was increased significantly by increasing the concentration of carbon dioxide from 400 to 800 ppm. Further increase in carbon dioxide to 1200 ppm did not increase the dry weight significantly over the 800 ppm concentration (Table 5).

Reducing the root size by 50 per cent had a slight effect on the dry weight of plants grown at the low concentration of carbon dioxide (400 ppm). However, at higher concentrations of carbon dioxide, removal of 50 per cent of the root system reduced significantly the dry weight of all plant parts (Table 6). The leaf area was drastically reduced by removal of 50 per cent of the root system, especially at higher concentrations of carbon dioxide. The ratio of leaf dry weight/leaf area was slightly higher for root pruned plants grown at a low concentration of carbon dioxide, but at a higher concentration of carbon dioxide this ratio was much larger for plants with a complete root system than for plants with pruned roots (Table 7 and Fig. 10).
Table 3. Influence of root size on plant leaf area and on leaf dry weight/leaf area ratio.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Weeks from planting</th>
<th>Leaf area (cm\textsuperscript{2} plant\textsuperscript{-1})</th>
<th>10\textsuperscript{-3} gDW/cm\textsuperscript{2} leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
<td>R75</td>
</tr>
<tr>
<td>4</td>
<td>830.7</td>
<td>871.0</td>
</tr>
<tr>
<td>5</td>
<td>1958.0</td>
<td>1608.7</td>
</tr>
<tr>
<td>6</td>
<td>2660.2</td>
<td>2345.5</td>
</tr>
<tr>
<td>7</td>
<td>3642.2</td>
<td>3159.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Statistical analysis of variance revealed no significant differences between the root treatments.

Table 4. Influence of root size on the net assimilation rate.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Weeks intervals</th>
<th>Net assimilation rate (mg cm\textsuperscript{2} day\textsuperscript{-1})\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
</tr>
<tr>
<td>4-5</td>
<td>0.537</td>
</tr>
<tr>
<td>5-6</td>
<td>0.520</td>
</tr>
<tr>
<td>6-7</td>
<td>0.689</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Statistical analysis is not applicable.

\textsuperscript{b}Net assimilation rate = \frac{\ln L_2 - \ln L_1}{L_2 - L_1} \times \frac{W_2 - W_1}{t_2 - t_1} (Watson 1952).
Table 5. Effect of carbon dioxide concentration on dry weight of the different plant parts.

<table>
<thead>
<tr>
<th>Part of the plant</th>
<th>400 p.p.m.</th>
<th>800 p.p.m.</th>
<th>1200 p.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>3.818a*</td>
<td>7.574b</td>
<td>7.976b</td>
</tr>
<tr>
<td>Stems + Petioles</td>
<td>2.377a</td>
<td>4.885b</td>
<td>4.716b</td>
</tr>
<tr>
<td>Shoot</td>
<td>6.196a</td>
<td>12.459b</td>
<td>12.692b</td>
</tr>
<tr>
<td>Root</td>
<td>1.527a</td>
<td>2.096b</td>
<td>2.299b</td>
</tr>
</tbody>
</table>

*Values within a row not followed by the same letter are significantly different at the 1% level of probability.

Table 6. Influence of root size on dry weight of the different parts of Red Kidney Bean plants grown under different concentrations of carbon dioxide.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Dry weight per plant (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>400 p.p.m.</td>
</tr>
<tr>
<td></td>
<td>R100</td>
</tr>
<tr>
<td>Leaves</td>
<td>4.22* 3.41</td>
</tr>
<tr>
<td>Stem + Petiole</td>
<td>2.56</td>
</tr>
<tr>
<td>Shoot</td>
<td>6.78* 5.61</td>
</tr>
<tr>
<td>Root</td>
<td>1.73* 1.33</td>
</tr>
</tbody>
</table>

*Indicates significant difference at 5% level of probability.

**Indicates significant difference at 1% level of probability.
Table 7. Influence of root size on the leaf area and leaf dry weight/leaf area ratio of Red Kidney Bean plants grown under different concentration of carbon dioxide.

<table>
<thead>
<tr>
<th>Concentration of carbon dioxide (p.p.m.)</th>
<th>Total leaf area (cm²)</th>
<th>$10^{-3}$ gDW/cm² leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
<td>R50</td>
</tr>
<tr>
<td></td>
<td>R100</td>
<td>R50</td>
</tr>
<tr>
<td>400</td>
<td>1620**</td>
<td>1247</td>
</tr>
<tr>
<td>800</td>
<td>2611*</td>
<td>1953</td>
</tr>
<tr>
<td>1200</td>
<td>2375</td>
<td>1863</td>
</tr>
</tbody>
</table>

*Indicates significant difference at 5% level of probability.

**Indicates significant difference at 1% level of probability.
Fig. 10. Effect of manipulation of root size on the leaf dry weight/leaf area ratio of plants grown under different concentrations of carbon dioxide.
Plants harvested at maturity show that removal of 50 per cent of the roots did not significantly affect the dry weight of pods or the total dry weight of roots (Table 8).

Table 8. Influence of root size on the dry weight of roots and pods of Red Kidney Bean plants at maturity stage grown under different concentrations of carbon dioxide.\(^a\)

<table>
<thead>
<tr>
<th>Concentrations of carbon dioxide (p.p.m.)</th>
<th>Dry weight of root (g)/plant</th>
<th>Dry weight of pods (g)/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
<td>R50</td>
</tr>
<tr>
<td>400</td>
<td>4.05</td>
<td>3.70</td>
</tr>
<tr>
<td>800</td>
<td>5.02</td>
<td>3.28</td>
</tr>
<tr>
<td>1200</td>
<td>4.11</td>
<td>4.45</td>
</tr>
</tbody>
</table>

\(^a\) Statistical analysis reveals no significant difference between root treatments.
Influence of Root Size on the Dry Weight of Pods and Roots of Plants Grown Under High Humidity and Different Temperatures

The effect of removing part of the roots on the dry weight of mature pods and on the total dry weight of roots is presented in Table 9. The findings show that whether the night temperature was 18 or 24 C, removal of 25 or 50 per cent of the roots, early or late at flowering, resulted in increase in both dry weight of roots and pods of plants grown under high humidity. While the increase in dry weight of pods was significant for plants grown under 24 C night temperature, it was not significant for plants grown under 18 C night temperature. This was largely due to the great variation among experimental plants. Total dry weight of roots was higher for pruned plants over plants with complete root systems, for all root treatments. Dry weight of pods and roots was higher for plants grown under low night temperature than for those grown under high temperature.

Plants grown under day/night temperature of 29 C failed completely to produce any mature pods, although, they were loaded with hundreds of small, 4-5 cm long immature pods. For this reason, results obtained from this part were excluded. This effect of continuous temperature of 29 C on fruiting of red kidney bean plants was confirmed by growing red kidney bean plants in a small growth chamber in which temperature was maintained continuously at 29 C. In that case also, numerous small pods were produced that did not mature,
Table 9. Influence of root size on the dry weight of pods and roots of plants grown under different night temperatures and high relative humidity.

<table>
<thead>
<tr>
<th>Treatments&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pods dry weight</th>
<th>Total root dry weight</th>
<th>Pods dry weight</th>
<th>Total root dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>R100</td>
<td>13.14a*</td>
<td>6.82a</td>
<td>18.06a</td>
<td>6.63a</td>
</tr>
<tr>
<td>R75 (1)</td>
<td>22.95b</td>
<td>9.97b</td>
<td>22.41a</td>
<td>11.17b</td>
</tr>
<tr>
<td>R50 (1)</td>
<td>18.07ab</td>
<td>9.91b</td>
<td>24.75a</td>
<td>11.10b</td>
</tr>
<tr>
<td>R75 (2)</td>
<td>21.12b</td>
<td>9.29b</td>
<td>28.41a</td>
<td>10.54b</td>
</tr>
<tr>
<td>R50 (2)</td>
<td>15.29ab</td>
<td>9.08b</td>
<td>24.04a</td>
<td>10.77b</td>
</tr>
</tbody>
</table>

<sup>a</sup>(1) Root pruning started when plants were 2 weeks old, (2) Root pruning started when plants began flowering.

*Values within a column not followed by the same letter are significantly different at the 1% level of probability.
Influence of Root Size on the Content of Ethanol Soluble Sugars in the Different Plant Parts

The effect of removing 50 per cent of the root on the content of ethanol soluble sugars in the different plant parts is presented in Table 10 and in Figs. 11 and 12. The results obtained showed that removing 50 per cent of the roots significantly increased the soluble sugars content in all parts of the plant. This was found to be the case whether the sugar content was expressed on weight or length basis.

Table 10. Influence of removing 50 per cent of the roots on the content of ethanol soluble sugar in the different plant parts.

<table>
<thead>
<tr>
<th>Part of the plant</th>
<th>Sugar content</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>μg sugar per gram fresh weight</td>
<td>μg sugar per centimeter length</td>
</tr>
<tr>
<td></td>
<td>R100</td>
<td>R50</td>
<td>R100</td>
</tr>
<tr>
<td>Leaf</td>
<td>13925</td>
<td>15662**</td>
<td>--</td>
</tr>
<tr>
<td>Petiole</td>
<td>2825</td>
<td>3413**</td>
<td>137</td>
</tr>
<tr>
<td>Upper stem</td>
<td>3191</td>
<td>3894**</td>
<td>229</td>
</tr>
<tr>
<td>Lower stem (a)</td>
<td>3668</td>
<td>4446**</td>
<td>406</td>
</tr>
<tr>
<td>Lower stem (b)</td>
<td>2147</td>
<td>2713**</td>
<td>274</td>
</tr>
</tbody>
</table>

aRefer to Fig. 3B.

** Indicates significant difference at 1% level of probability.
Fig. 11. Effect of manipulation of root size on the content of soluble sugars (μg cm⁻¹) in the different parts of the simplified plant.
Fig. 12. Effect of manipulation of root size on the content of soluble sugars (μg g⁻¹) in the different parts of the simplified plant.
While removing 50 per cent of the roots increased the concentration of soluble sugars in the different plant parts, it did not alter the sugar concentration gradient down the stem (Fig. 11). This gradient was expressed as the difference in sugar content per centimeter between two points over the distance between them or \( \frac{\Delta C}{\Delta x} \). The removal of 50 per cent of the roots did not change the sugar content ratio when this ratio was expressed as \( (\mu g \text{ sugar g}^{-1} \text{ lower stem})/(\mu g \text{ sugar g}^{-1} \text{ upper stem}) \) either (Fig. 12).

**Movement of Sucrose C-14**

The movement of exogenously applied sucrose C-14 from a fully expanded leaflet to other plant parts was followed autoradiographically in simplified plants after 15, 30, 60, 120, 240, and 480 minutes from the time of sucrose application. The results obtained did not clearly demonstrate any difference, in regard to the movement of sucrose C-14, between plants with complete root systems and plants with half of their roots removed. In a few cases, root pruning resulted in slight activity in the upward direction, but this is by no means absolute, Fig. 13a and 13b. However, the results clearly demonstrate the following:

1. The vein loading process of sucrose C-14 proceeded from the site of application to the nearest main veins via some minor veins (Fig. 14).

2. Movement of the labelled sucrose in the source leaf was ultimately basipetally, toward the base of the leaflet. No cross-over movement of the label was detected from one half of the blade into the other half (Fig. 14).
Fig. 13. Autoradiograms showing the effect of manipulation of root size on the movement of sucrose C-14, one hour after application -- (A) R100, (B) R50.
Fig. 14. Autoradiogram of the movement of sucrose C-14 from the source leaf, after 30 minutes -- Notice the entrance of the label to the minor veins first and then the basipetal movement into the major veins.
3. The bulk of the translocated labelled sucrose was moved mainly down the stem, toward the roots (Fig. 13).

Translocation Velocity of Labelled Photosynthate

The relative translocation velocities of labelled photosynthate from a source leaf toward the upper and lower sinks were measured by plotting the transformed percentage of the total radioactivity in the successive one centimeter sections of the stem. It was found that transformed percentage of radioactivity vs. distance from the source followed a highly significant, linear relationship. Therefore, comparison of the slopes of the regression lines was a valid parameter for measuring the relative translocation velocities (Nelson et al., 1961). The results obtained in this experiment are graphically shown in Fig. 15. The findings indicate the following:

1. The upward translocation velocity. After a 15 minute translocation period, following a 10 minute exposure to labelled $CO_2$, the plants with a complete root system (R100) demonstrated a negative slope of $b = -5.16$. It was $b = -10.7$ for plants with the roots pruned (R50) (Fig. 15a). The plants removed after a 30 minute translocation period demonstrated slopes of $b = +1.16$ and $b = -9.8$ for R100 and R50, respectively (Fig. 15b). These slopes were significantly different from each other. It can also be seen that, with time, while the slope of the R50 plants was slightly increased from -10.7 to -9.8, the slope was much increased for R100 plants and it was already positive after 30 minutes. It increased from -5.16 to +1.16. It is evident from
Fig. 15. Regression for radioactivity vs. distance for measuring the relative translocation velocity of C-14 -- (A) 15 min, (B) 30 min, (C) 15 min, and (D) 30 min. ** indicates significant difference at 1% level of probability.
these findings that removal of 50 per cent of the roots significantly reduced the upward translocation velocity of labelled photosynthate.

2. The downward translocation velocity. Results of translocation velocity of labelled photosynthate toward the roots is shown in Fig. 15c and 15d. Comparison of the slopes of the regression lines revealed that the differences were not significant. This was found to be the case after the 15 and 30 minute translocation periods. However, the slopes increased with time and became less negative. This was obviously due to the further movement of the radioactive fronts. The results indicated that removing 50 per cent of the roots did not change the downward translocation velocity of the labelled photosynthate.

Influence of Root Size on Distribution of Labelled Photosynthate in Simplified Plants

The influence of root size on the distribution of the ethanol soluble labelled photosynthate in the different parts of simplified plants is shown in Table 11. The results obtained show that removing 50 per cent of the roots, although not changing the total amount of labelled photosynthate exported from the source, did change significantly the pattern of their distribution. While more than 59 per cent of the total exported labelled photosynthate moved toward the roots of plants with a complete root system, it was only 30 per cent for root pruned plants. The percentage of labelled photosynthate exported to the upper stem was 35 for R100 plants, and it was 52 for R50 plants.
Table 11. Influence of root size on the distribution of ethanol soluble radioactive photosynthate in the different plant parts.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>R100</th>
<th>R50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.P.M.</td>
<td>% total</td>
</tr>
<tr>
<td>Source leaf</td>
<td>$3.5 \times 10^5$</td>
<td>77.1a*</td>
</tr>
<tr>
<td>Petiole</td>
<td>$1.3 \times 10^5$</td>
<td>2.8a</td>
</tr>
<tr>
<td>Upper stem</td>
<td>$3.0 \times 10^5$</td>
<td>6.5a</td>
</tr>
<tr>
<td>Lower stem</td>
<td>$5.5 \times 10^5$</td>
<td>12.1a</td>
</tr>
<tr>
<td>Roots</td>
<td>$6.9 \times 10^4$</td>
<td>1.5a</td>
</tr>
<tr>
<td>Total Export a</td>
<td>$1.1 \times 10^6$</td>
<td>22.9a</td>
</tr>
<tr>
<td>Distribution ratio b</td>
<td>2.263a</td>
<td></td>
</tr>
</tbody>
</table>

*Values within a row not followed by the same letter are significantly different at 5% level of probability.

a Total export = Total Radioactivity of plant radioactivity of source leaf (Mortimer 1965).

b Distribution ratio = (Radioactivity of lower stem + roots)/(Radioactivity of upper stem) (Thrower 1962).

Because the total exported labelled photosynthate was similar for both R100 and R50 plants, the distribution ratio was a valid parameter to use in assessing the influence of root pruning on the distribution of photosynthate. This ratio was found to be significantly reduced by removing 50 per cent of the roots (Table 11).

The effect of removing 50 percent of the roots on distribution of the radioactivity in the main three sugars (sucrose, glucose, and fructose) is summarized in Table 12. The findings show that, among the three sugars investigated, the radioactivity was mainly in sucrose, with
Table 12. Influence of root size on percentage of radioactivity present in sucrose, glucose, and fructose of the different plant parts.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Sucrose</th>
<th>Glucose</th>
<th>Fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source leaf</td>
<td>64.6</td>
<td>17.2</td>
<td>18.2</td>
<td>74.9*</td>
<td>12.3</td>
<td>12.8</td>
</tr>
<tr>
<td>Petiole</td>
<td>75.3</td>
<td>12.8</td>
<td>11.9</td>
<td>79.3</td>
<td>11.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Upper stem</td>
<td>61.0</td>
<td>18.1</td>
<td>20.9</td>
<td>61.2</td>
<td>21.1</td>
<td>17.7</td>
</tr>
<tr>
<td>Lower stem</td>
<td>90.0</td>
<td>5.0</td>
<td>5.0</td>
<td>90.9</td>
<td>5.2</td>
<td>3.9</td>
</tr>
</tbody>
</table>

*Only the sucrose percentage in the source leaf of R50 plants was significantly higher than in R100 plants at 5% level of probability.

the highest in the lower stem and the lowest in the upper stem.

Radioactivity of glucose and fructose was very similar and close to a 1:1 ratio. The partial partial derootment of the plant increased significantly the percentage of sucrose activity of the source leaf. Distribution of radioactivity among the three sugars in the other plant parts was found not affected by the root treatment.

Influence of Root Size on the Distribution Pattern of Photosynthetically Assimilated C-14 in Complete Plant System

The distribution of C-14 in the different plant parts, 6 hours after $^{14}C_2O_2$ assimilation for 10 minutes by the second trifoliate, is given in Table 13. The data were computed to present the radioactivity in the alcohol soluble and in the insoluble fractions as percentages of the total radioactivity incorporated by the plant. In this way the
Table 13. Distribution of C-14 in the different plant parts 6 hours after $^{14}CO_2$ assimilation for 10 min by the second trifoliate.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>R100 Soluble</th>
<th>R100 Insoluble</th>
<th>R100 Total</th>
<th>R50 Soluble</th>
<th>R50 Insoluble</th>
<th>R50 Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.007</td>
<td>0.117</td>
<td>0.124</td>
<td>0.013</td>
<td>0.148</td>
<td>0.161</td>
</tr>
<tr>
<td>L1</td>
<td>0.020</td>
<td>0.186</td>
<td>0.206</td>
<td>0.021</td>
<td>0.151</td>
<td>0.172</td>
</tr>
<tr>
<td>L2C</td>
<td>7.805</td>
<td>67.220</td>
<td>75.025</td>
<td>8.808</td>
<td>68.186</td>
<td>76.994</td>
</tr>
<tr>
<td>L3</td>
<td>0.068</td>
<td>1.940</td>
<td>2.008</td>
<td>0.067</td>
<td>1.826</td>
<td>1.893</td>
</tr>
<tr>
<td>L4</td>
<td>0.234</td>
<td>1.512</td>
<td>1.746</td>
<td>0.073</td>
<td>0.367</td>
<td>0.440</td>
</tr>
<tr>
<td>S1</td>
<td>0.340</td>
<td>4.499</td>
<td>4.839</td>
<td>0.766</td>
<td>4.965</td>
<td>5.731</td>
</tr>
<tr>
<td>S2</td>
<td>0.051</td>
<td>1.602</td>
<td>1.653</td>
<td>0.102</td>
<td>2.056</td>
<td>2.158</td>
</tr>
<tr>
<td>S3</td>
<td>0.104</td>
<td>3.241</td>
<td>3.345</td>
<td>0.214</td>
<td>3.732</td>
<td>3.946</td>
</tr>
<tr>
<td>S4</td>
<td>0.108</td>
<td>1.915</td>
<td>2.023</td>
<td>0.241</td>
<td>1.430</td>
<td>1.671</td>
</tr>
<tr>
<td>S5</td>
<td>0.414</td>
<td>2.307</td>
<td>2.721</td>
<td>0.445</td>
<td>2.013</td>
<td>2.458</td>
</tr>
<tr>
<td>R</td>
<td>1.694</td>
<td>4.616</td>
<td>6.310</td>
<td>1.110</td>
<td>3.266</td>
<td>4.376</td>
</tr>
<tr>
<td>Total</td>
<td>10.845</td>
<td>89.155</td>
<td>100.000</td>
<td>11.860</td>
<td>88.140</td>
<td>100.000</td>
</tr>
</tbody>
</table>

Distribution Ratio$^d$ 2.69 1.86 1.94 2.69 2.54 2.56

Export -- -- 24.975 -- -- 24.006

$^a$ Analysis of variance revealed no significant difference between root treatments.

$^b$ Refers to Fig. 6 for meaning of the symbols.

$^c$ Leaf that received the radioactive carbon dioxide.

$^d$ Distribution Ratio = $\frac{P + L_1 + S_1 + S_2 + S_3 + R}{L_3 + L_4 + S_4 + S_5}$
radioactivities in the soluble and insoluble fractions for the different plant parts are readily compared.

Regardless of the influence of manipulation of the root size on the distribution pattern, the data obtained in this experiment show the following general pattern:

1. Most of the radioactivity, about 90%, was found in the insoluble form.
2. Mature leaves such as the primary and the first trifoliate imported very little assimilate from the above source leaf.
3. The source leaf retained as much as 75% of the total assimilated C-14. This was found mostly in the insoluble form.
4. The roots and the lower stem were the major importing organs, followed by the upper plant parts.

In regard to the influence of removing 50 per cent of the roots on the distribution of the assimilated C-14 in the different plant parts, the results obtained show the following:

1. The manipulated roots imported less assimilated C-14 than complete root systems.
2. The total export from the source leaf was not significantly affected by the partial derootment.
3. Export to the upper plant parts such as L₂, L₄, S₄, and S₅ was reduced by removing 50 per cent of the roots. However, the reverse was true for export to the lower plant parts, in particular, S₁, S₂, and S₃. The partial derootment increased the percentage of activity of these parts.
4. While manipulation of root size did not alter the distribution ratio in the ethanol soluble fraction, it did increase the ratio for the insoluble fraction.

In summary, removing 50 per cent of the roots did not alter the total export from the source. However, it resulted in more movement or incorporation of the translocated assimilate in the lower stem portions with less movement or incorporation in the upper plant parts.

Influence of Manipulation of Root Size and Kinetin Spray on the Fresh and Dry Weights of the Different Plant Parts

The results of this 2x2 factorial experiment are shown in Tables 14 and 15. It appears from the results obtained that without Kinetin spray, removal of 50 per cent of the roots reduced both the fresh and dry weights of the different plant parts, significantly the fresh weights of the shoot and pods. However, for plants sprayed with Kinetin, the removal of 50 per cent of the roots did not have any depressing effect on the fresh or dry weights of the different plant parts. On the contrary, it increased slightly the fresh weight of pods over that of plants with complete root system sprayed also with Kinetin.

Because root pruning decreased significantly the fresh weight of pods in the non-sprayed plants and because spraying with Kinetin increased significantly the fresh weight of pods of root-pruned plants not sprayed, the interaction effect between Kinetin and root pruning was subjected to statistical analysis of variance. The results obtained showed that there was significant interaction between these two factors at 10% level of probability \(F = 4.694, \text{ d.f.} = 1 \text{ and } 12 \text{ and S.E.} = \)
Table 14. Influence of manipulation of root size and kinetin spray on the fresh weight of the different plant parts.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>No spray</th>
<th>Sprayed with kinetin (10 ppm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
<td>R50</td>
<td>R100</td>
<td>R50</td>
</tr>
<tr>
<td>Leaves</td>
<td>45.4a*</td>
<td>31.7a</td>
<td>36.2a</td>
<td>35.2a</td>
</tr>
<tr>
<td>Stem</td>
<td>22.9a</td>
<td>17.1a</td>
<td>19.9a</td>
<td>18.8a</td>
</tr>
<tr>
<td>Pods</td>
<td>69.9a</td>
<td>42.5b</td>
<td>58.6ab</td>
<td>60.8a</td>
</tr>
<tr>
<td>Shoot</td>
<td>138.1a</td>
<td>91.3b</td>
<td>114.6ab</td>
<td>114.9ab</td>
</tr>
</tbody>
</table>

*Values within a row not followed by the same letter are significantly different at 5% level of probability.

Table 15. Influence of manipulation of root size and kinetin spray on the dry weight of the different plant parts.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>No spray</th>
<th>Sprayed with kinetin (10 ppm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R100</td>
<td>R50</td>
<td>R100</td>
<td>R50</td>
</tr>
<tr>
<td>Leaves</td>
<td>6.12</td>
<td>4.20</td>
<td>4.60</td>
<td>4.44</td>
</tr>
<tr>
<td>Stem</td>
<td>4.16</td>
<td>2.88</td>
<td>3.34</td>
<td>3.18</td>
</tr>
<tr>
<td>Pods</td>
<td>7.24</td>
<td>4.70</td>
<td>5.60</td>
<td>5.96</td>
</tr>
<tr>
<td>Shoot</td>
<td>17.54</td>
<td>11.78</td>
<td>13.54</td>
<td>13.60</td>
</tr>
<tr>
<td>Root</td>
<td>2.26</td>
<td>0.94</td>
<td>1.60</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Statistical analysis reveals no significant differences among the different treatments.
±6.85). The data obtained from the dry weight measurements showed basically the same trend. However, the difference between the treatments was not statistically significant at the acceptable levels of probabilities.

Nevertheless, the results of this experiment suggested that removing 50 per cent of the roots reduced the growth of the different plant parts, and spraying the foliage with Kinetin removed most, if not all, of this effect.

Influence of Removing 50 Per Cent of the Roots on the Photosynthetic Capacity of the Source-Leaf in Simplified Plant Systems

The data obtained from measurement of the rates of carbon dioxide uptake are presented in Table 16. The results show that removal of 50 per cent of the roots of Red Kidney bean plants did not significantly affect the photosynthetic capacity of the source leaf during the 72 hours after the root pruning treatment.
Table 16. Influence of removal of 50 per cent of the roots on the photosynthetic capacity of the source leaf in simplified plant systems.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Time (hours)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24</td>
<td>48</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Leaf area (dm²)</td>
<td>R100</td>
<td>R100</td>
<td>R50</td>
<td>R50</td>
</tr>
<tr>
<td></td>
<td>2.07</td>
<td>2.10</td>
<td>1.88</td>
<td>1.76</td>
</tr>
<tr>
<td>ΔCO₂ Conc.⁴ p.p.m.</td>
<td>265</td>
<td>340</td>
<td>300</td>
<td>345</td>
</tr>
<tr>
<td>Net photosynthetic rate (mg CO₂ dm⁻² hr⁻¹)⁵</td>
<td>8.05</td>
<td>10.18</td>
<td>10.03</td>
<td>12.32</td>
</tr>
</tbody>
</table>

⁴ΔCO₂: change in the concentration of carbon dioxide in the system during 30 minutes.

⁵Differences between root treatments were not significant.
DISCUSSION

Besides anchoring plants into the soil, roots function primarily in absorption of water and nutrients, substances that are required for growth and development of the whole plant. In addition, roots of plants seem to offer sites for metabolism of some organic acids (Kursanov 1957) and for synthesis of some growth hormones (Mothes and Engelbert 1963; Carr, Reid, and Skene 1964; Kende 1965). Because roots form a large volume of non-photosynthesizing living tissues, they also represent a strong sink for photosynthate that is produced solely by the shoot. Whether the plants need all of their root system or not is an open question. The objective of this study was not to answer this question, but to examine the effect of manipulating the size of this sink on the dynamics of photosynthate translocation. In order to do this, it was necessary to remove as much as possible of the roots without altering significantly some of the physiological functions of the root, such as absorption of water and nutrients. The results presented in Table 1 and Fig. 9 indicated that the relative water content of the leaves was decreased as more roots were removed. However, removal of up to 50 per cent of the roots did not significantly change the water status of the plant. This suggested that the 50 per cent root-pruned plants were not suffering from inadequate water supply. As for nutrient uptake, the experimental plants looked healthy and no signs of nutrient deficiency were observed. These results agreed with the findings of Buttrose and Mullins (1968) on grape vines.
Cutting down the sink size of roots by removing 25 or 50 per cent of the roots had little influence on partitioning of the dry matter in the different plant parts, including the roots (Table 2). Similar results were obtained by Humphries (1958) and Cooper (1971).

In order to see if accumulation of dry matter took place, especially in the leaves, the leaf dry weight/leaf area ratio was determined (Burt 1964). Removing 25 or 50 per cent increased insignificantly this ratio. However, the reduction in the leaf area was greater than could be accounted for by the slight increase in the dry weight/leaf area ratio. This suggested that the partial derootment affected directly the growth rate of the leaves. The mode of this effect may lie in cell division or expansion, however availability of photosynthate is unlikely to be the limiting factor since the leaf dry weight/leaf area ratio was similar to that of control plants. Computation of net assimilation rate (Table 4) gave no indication that root treatment altered the assimilation capacity of the leaves, since net assimilation rate was expressed as dry weight increment per unit leaf area per unit time.

Many studies, including those of Mortimer (1959), demonstrated that rate of assimilation was increased in proportion to the increase in concentration of the surrounding carbon dioxide. Therefore, an attempt was made to investigate how much the root size would limit growth of plants grown under different levels of carbon dioxide. The results given in Table 5 show that increased concentrations of carbon dioxide significantly increased the dry weight of the different plant parts. Focusing then on the effect of root size on growth of plants grown under these different conditions (Table 6), it was revealed that reduction of
root size by pruning significantly decreased the dry weight of the different plant parts, and this reduction was proportionately much greater under higher concentration of ambient carbon dioxide than when the concentration of the ambient carbon dioxide was close to the atmospheric concentration, about 400 ppm. The partial derootment also reduced significantly the leaf area (Table 7). Leaf dry weight/leaf area ratio (Table 7 and Fig. 10) showed an interesting trend. The effect of root treatment on this ratio for plants grown under the lowest concentration of carbon dioxide was similar to that obtained from plants grown under atmospheric concentration of carbon dioxide (Table 3). The increased concentrations of carbon dioxide, however, increased this ratio for plants with complete root systems over plants with 50 per cent of their roots removed. This suggested that reduction in root size not only decreased leaf expansion but under stimulatory conditions for photosynthesis, reduction in root size decreased the photosynthetic or mobilization capacity of the leaves.

Under high relative humidity conditions, removing 25 or 50 per cent of the root systems increased significantly the total dry weight of roots over the control (Table 9). The increasing effect of the partial derootment on the dry weight of pods was also evident. Because of the different effect of root pruning, in comparison to results obtained from previous experiments, on the total dry weight of roots, the data suggest that root pruning stimulated root growth under conditions of high relative humidity, and the high dry weight of pods was obtained as a result of this high root growth. The exact nature of the stimulatory effect of the high relative humidity on the growth of
roots is not clear. However, it appears that high humidity conditions
decreased the transpiration rate (O'Leary and Knecht 1971), consequently
possibly decreasing the upward movement of absorbed nutrients as well as
the delivery of metabolites synthesized in the roots such as organic
acids or some hormones. The high relative humidity condition also
might have increased translocation of photosynthate (Clor et al. 1962).
Accumulation of these compounds along with the availability of photo­
synthate may have resulted in stimulation of root growth and in lateral
root formation especially in the pruned roots. Since synthesis of
these hormones apparently takes place in the root tips (Jones and
Phillips 1966) the level of the hormones might have been higher in the
root-pruned plants than in the control plants. The increase in the dry
weight of roots and pods of the root-pruned plants apparently was due to
the availability of both photosynthate and hormones, because when the
sink size was reduced, photosynthate was more available for other sinks
as well as for the root itself. Confirmation of this conclusion came
from the effect of night temperature on the dry weight of roots and pods
(Table 9). Root-pruned plants grown under low night temperature (18 C)
produced higher dry weight for roots and pods than for plants grown
under the higher night temperature (24 C). Apparently under the low
temperature condition the respiration rate was less than that at the
high temperature, which means more assimilates were available for growth
in the former condition than in the latter, and this resulted in the
higher dry weight of roots and pods obtained.

Availability of root hormones appears to be a requirement for
normal shoot growth. Root-pruned plants grown under high concentration
of carbon dioxide, but low relative humidity condition, produced low dry weight for roots and shoots. They failed to benefit from the high concentration of carbon dioxide. Under such conditions, deficiency in root hormones apparently reduced the leaf dry weight/leaf area ratio as well as the total leaf area. This indicates the importance of such hormones in the expansion of leaves and on their mobilization power.

Manipulation of sink size increased significantly the level of alcohol soluble sugars in the different plant parts (Table 10, Fig. 11, Fig. 12). These findings validate the assumption that reducing the sink size of roots would result in more available photosynthate. However, it did not show preferential distribution of the soluble sugars since the ratio of sugar content remained unchanged (Fig. 12). Manipulation of root size, although it increased the concentration of the soluble sugars, did not change their concentration gradient down the stem (Fig. 11). These results suggested that reducing the sink size increased the soluble sugar content uniformly in the different parts of the plant, but by no means should one take this as an indication of the distribution of the total sugars, soluble plus insoluble, because the partial derootment appeared to influence unequally the metabolic activity of the different plant parts.

Measurements of the relative translocation velocity of labelled photosynthate demonstrated that manipulation of root size significantly decreased the upward movement toward the developing fruits, but it did not change the downward movement toward the roots (Fig. 15). These results are readily explainable by the assumption that roots supply the shoot organs with hormone-like compounds required for maintaining the
mobilization power of these organs. It appears, therefore, that cutting down the size of roots significantly retarded the delivery of these hormones to the developing fruits. Consequently, they lost most of their ability for utilizing the excess carbohydrates. This may then result in accumulation of sugars in the upper stem and the sugar concentration gradient was lost, and consequently the velocity of the labelled photosynthate was significantly reduced toward this sink. This explanation is in line with the views of Hew et al. (1967) who showed that plant hormones control the rate of translocation of assimilates as well as the total amount translocated. On the other hand, the translocation velocity of the labelled photosynthate toward the roots was not altered by removing 50 per cent of the roots. Apparently, the mobilization power of the remaining roots was not seriously affected. This is probably due to the ability of the remaining roots to satisfy their own needs from these hormones before any are exported away to the shoot. Therefore, they were able to maintain the downward sugar concentration gradient (Fig. 11). This results in similar translocation velocity to that occurring in the control plants.

The autoradiographic study, using exogenously applied sucrose C-14, did not reveal any distinguishing visual differences in the movement of the label between plants with complete root systems vs. plants with half of their roots removed. The reason for this is not clear. However, autoradiography is a technique that is subject to many limitations. Nevertheless, the results obtained show that the bulk of the label was moved downward in the stem toward the roots. This is in agreement with the findings of Nakata and Leopold (1967).
The distribution pattern of the photosynthetically labelled assimilate in a simplified plant system was drastically changed by removing 50 per cent of the roots. Manipulation of the root size reduced significantly the amount of labelled assimilates that moved toward the roots and increased the amount moved toward the expanding leaf (Table 11). It may appear that these results do not agree with the results obtained in other experiments, such as the one reported for translocation velocity, in which cutting down the root size significantly reduced the upward movement. This discrepancy apparently was the result of the differences in nature of the upper sinks used in each experiment. It was the 8-10 developing pods in the translocation velocity experiment, but it was the small expanding fourth trifoliate in this experiment. The developing fruits form a much stronger sink (Hale and Weaver 1962, Hansen 1967) than the single expanding trifoliate. Although cutting down 50 per cent of the roots would reduce the amount of hormones delivered to the top for both cases, this amount was apparently sufficient to maintain the mobilizing power of the small expanding trifoliate and not for the massive 8-10 developing pods. Therefore, the movement of labelled assimilates was reduced toward the developing pods and it was not affected toward the expanding leaf. The results obtained from the simplified plant system support strongly the view that distribution of photosynthate is controlled by the supply and demand concept. Cutting down sink size significantly reduced the amount of assimilates moved toward that sink, and it resulted in higher distribution of photosynthate to the other sinks as long as the mobilization capacity of these other sinks was not handicapped. Because the total
export of labelled assimilate from the source leaf was not affected by the partial derootment (Table 11), the distribution ratio is a valid estimate for evaluating the influence of the treatment on the distribution of the photosynthate. This ratio was found to be significantly reduced by the removal of 50 per cent of the roots, indicating that partial derootment significantly altered distribution of photosynthate in simplified plant systems. These results are in agreement with results obtained by Wardlaw (1968) who found that the distribution pattern of assimilated C-14 was changed upon removing developing grains from the head of wheat. Also, the rate of movement out of the source leaf was unaffected by the manipulation of this sink.

The effect of root size on the incorporation of C-14 in the three main sugars, sucrose, glucose, and fructose, was investigated. Results in Table 12 showed that sucrose is the main form of translocated sugar in Red Kidney beans. This is in agreement with the general view that sucrose is the common translocated sugar form in many plants. Incorporation of C-14 into glucose and fructose was also detected, and they were found in a ratio close to 1:1. Results similar to this were obtained by Swanson and El-Shishiny (1958) in concord grapes. Removing 50 per cent of the roots did not significantly alter the relative percentages of C-14 found in these three sugar forms in the different plant parts. Exception to this was found in the source leaf which showed that the partial derootment significantly increased C-14 in sucrose relative to the other two sugars. No clear explanation for this was available due largely to lack of information concerning the biochemical steps
involved in the vein loading process from the mesophyll cell into the phloem elements of the leaf.

Distribution of photosynthetically assimilated C-14 in complete plant systems, 6 hours after C\textsuperscript{14}O\textsubscript{2} assimilation for 10 minutes by the second trifoliate, showed that most of the activity was incorporated in the ethanol insoluble fraction (Table 13). Apparently 6 hours is long enough for fixation of most of C-14 into the insoluble forms. Although the partial derootment did not significantly affect the total export of assimilated C-14 from the source leaf, it reduced the amount moved to the roots. While a complete root system incorporated about 25% of the total C-14 exported, it was 18% for the manipulated roots. As was expected, the upper parts of the plant, above the source leaf, incorporated less C-14 in the root pruned plants than in the control plants. This is very evident for the upper most expanding leaf, L\textsubscript{4} (Table 13). Because manipulation of root size decreased the amount of C-14 incorporated in the roots as well as in the upper stem parts and the total export was unaffected, most of this remaining assimilated C-14 was found to be incorporated in the lower stem. Manipulation of root size increased the distribution ratio of the C-14 incorporated in the insoluble fraction, but not in the soluble fraction, indicating that it increased the metabolic conversion of the assimilated C-14 in the lower plant parts or decreased this conversion in the upper plant parts, or both, which in all cases closely can be related to the mobility of these parts and to the preferential distribution of the exported assimilates to these parts. This alteration in the pattern of distribution of photosynthate again suggested the role of roots in
distribution of the translocated photosynthate to the upper plant parts through controlling the mobilizing power of these parts, probably by hormonal means. Removing 50 per cent of the roots apparently reduced the hormone delivery to the uppermost plant parts and consequently the translocation of photosynthate toward them was reduced.

Although the exact nature of the root hormones is not known, there are some evidences that they are Kinetin-like compounds (Mothes and Engelbert 1963, Kende 1965) or gibberellin-like compounds (Carr et al, 1964). Therefore, if the general suggestion that manipulation of root size would cut down the delivery of such hormones to the shoot, is correct, then spraying the foliage with such hormone-like compounds should remove some of this deficiency and maintain the mobilizing power of these parts. The results of such attempts (Tables 14 and 15) showed that the daily spray of foliage with 10 ppm solution of Kinetin increased the fresh and the dry weight of the different plant parts to levels similar to that of plants having complete root systems. In addition, the significant interaction between the root treatment and the Kinetin spray treatment strengthened the suggested mode of action for manipulation of the root size, that it reduced the mobilization power of other sinks by depriving them of the root hormones. Support for this comes from the recent findings of McDavid, Sagar, and Marshall (1973) that the supply of cytokinins from the roots to shoots may affect leaves by maintaining their capacity for photosynthesis.

It was shown that manipulation of root size increased the soluble sugar content in the different parts of the plants (Table 10). Maintaining the size of the roots at this level would be expected to
maintain this increase and the accumulation of these sugars should result in increases in the dry weight of the root-pruned plants as long as this accumulation does not affect the net photosynthetic rate. The results obtained (Table 3) showed that the partial derootment did not significantly increase the leaves g/cm². Measurement of the net photosynthesis rate using a simplified plant system (Table 16) revealed that the photosynthetic capacity of the source leaf was not affected.

Obviously this system can not continue in this fashion and must reach a point where the level of the accumulated photosynthate would stimulate feed-back inhibition mechanisms which finally should reduce the photosynthetic capacity of the source leaves. Whether this mechanism is controlled by the level of dry matter accumulated (Burt 1964, 1966) or by reducing the rate of translocation from the source leaf (Humphries 1963, Humphries and Thorne 1964), it must finally reduce the net photosynthetic rate. The rate of translocation from the source leaf, measured as total export (Table 11) was found to be unaffected by the partial derootment. Therefore the increase in the soluble sugar content noticed was apparently far from the level that would stimulate the suggested feed-back inhibition mechanism. It is unlikely, in simplified plants having only one source leaf that may hardly satisfy the requirements of photosynthate by the remaining sinks, that sufficient amounts of carbohydrates would be accumulated to stimulate the feed-back inhibition mechanism. Although using simplified plants did not make clear the influence of carbohydrate accumulation on the net photosynthetic rate, the results provide solid evidence that the photosynthetic
capacity of the single source leaf or the simplified plant, used throughout this study, was not affected.
CONCLUSION

The dynamics of photosynthate translocation in *Phaseolus vulgaris* plants were altered by the manipulation of sink size. The lines of this alteration followed in principle the concept of supply and demand. Although the manipulation of sink size did not affect the total export of photosynthate from the source leaf it did change their final distribution with less photosynthate moved to the manipulated sink. Because the manipulated sink, in this study, was the root which was also a source for some plant hormones required by the shoot, manipulation of this sink apparently reduced the strength of the other sinks on the shoot. Subsequently, the translocation velocity of photosynthates as well as their distribution were reduced toward these other sinks. The excess of photosynthate gained from manipulation of the roots was accumulated in the lower parts of the stem. It appears, therefore, that the final distribution pattern of photosynthate was the net result of these two interrelated processes. The alteration in the dynamics of photosynthate translocation in this manner, is probably of some survival value to the whole plant, to assure balanced ratio between the shoot and root. Reducing the root size would depress the growth of the shoot. Any beneficial effect from manipulation of the roots, as a major sink, is conditioned by maintaining the mobilization power of the shoot parts. This could be achieved, in part, by supplying the shoot with root hormone-like compounds.
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