Studies on Translocation of Photosynthetic Products in Young Soybean Plants Using $^{14}CO_2$ and $^{3}H_2O$

by

R. G. Thompson

A thesis submitted to the Department of Biological Sciences in conformity with the requirements for the degree of Master of Science

Simon Fraser University
Burnaby, British Columbia, Canada

June, 1966

Record of Thesis Defence

C. D. NELSON
Senior Supervisor

W. Vidaver
Supervising Committee

F. J. F. Fisher
Supervising Committee

Abstract

Glycine max L. variety Comet was grown under constant conditions of mineral nutrition and environment for 14, 19 and 24 days. When weights, leaf areas, and rate of photosynthesis were used as an index of growth and physiological development, the plants within each age group showed little variation. The total ^{14}C in the ethanol-soluble fraction of a fed primary leaf and the total ^{14}C translocated were measured after thirty minutes during which $^{14}CO_2$ was photosynthetically fixed in the primary leaf. Since 24-day-old plants translocated in only three out of five cases, they were discarded as material for short term translocation studies. Under conditions of non-steady-state photosynthesis, the total ^{14}C translocated is not correlated to the rate of photosynthesis or to the total ^{14}C in the fed leaf.

The total ^{3}H in the ethanol-soluble fraction of the fed leaf and the total ^{3}H translocated were measured after thirty minutes during which $^{3}H_2O$ was photosynthetically fixed in the primary leaf. The compounds in the ethanol-soluble fraction of the fed leaf labelled in the same pattern whether ^{14}C or ^{3}H was fed. However, ^{14}C was translocated as ^{14}C-sucrose while ^{3}H was not translocated in the form of sucrose but probably in the form of amino acids. Also, ^{14}C was translocated mainly up and down the stem, while ^{3}H was translocated mainly into the opposite leaf.

A new technique is described for assaying ^{3}H labelled compounds taken from paper chromatograms.

Acknowledgement

Dr. C. D. Nelson helped and encouraged me during the course of this work at Queen's University and later at Simon Fraser University. He suggested the problem, and assisted in the interpretation of the data and writing of the thesis. To Dr. Nelson I wish to express my thanks and appreciation.

Dr. G. Krotkov who permitted me to use the facilities and space of the Isotope Laboratory at Queen's University to undertake this research will always be remembered with appreciation. From him and Dr. Nelson, I have received generously their principles and philosophy of research in Plant Physiology.

To Mr. Choy-Sin Hew and Mr. G. R. Lister who taught me the techniques of isotope work, to Miss Elaine Walker who allowed me to use her data, to my fellow students and professors at Queen's and Simon Fraser Universities who discussed, criticized and encouraged my work, to Miss Agnes Medley who could always supply the equipment needed, and to Miss Jenny Turner and Miss Barbara Pizzuto who typed this thesis, I am indeed grateful.

Table of Contents

List of Tables

Page

Appendix Tables

Page

Introduction

Plants translocate organic materials, minerals and water to supply the substrate and energy in various parts of the plant for the complex processes of growth and differentiation. Radioactive isotopes are used to follow these movements. Organic materials labelled with radioactive isotopes can be fed to the plant through the leaves or stems, or the plant can incorporate radioactive isotopes into the organic compounds through the process of photosynthesis in the presence of $^{14}CO_2$ or $^{3}H_2O$. The movement of minerals is followed by using radioactive isotopes added to the water surrounding the roots. The water stream is labelled with $^{3}H_2O$ or ions soluble in water such as $^{32}PO_4^{-3}$ which is carried along in the water stream. After a period of time is allowed for translocation, the position of the tracer in the plant is determined by cutting the plant into sections and measuring the radioactivity present in each section. The labelled compounds are separated and identified by chromatography and the amount of activity in each compound is measured.

Translocation of radioisotopes has been studied extensively in relatively few plant species. The soybean one of these species, grows rapidly under conventional conditions, maturing within a few weeks to a plant which can be used to demonstrate translocation. Soybean has been used so exstensively that a considerable amount is known about the translocation

process in this plant. Also, the chemistry and biochemistry of soybean have been well documented leading to the publication of a monograph in 1963 (22). For these reasons, soybean was picked for the experiments described in this thesis

The translocation of compounds labelled with isotopic carbon is well documented for soybean. The technique of introducing ^{14}C-compounds into the cut petiole of a young primary leaf was used to study translocation of sugars (16) and amino acids (19) (20). These compounds were shown to move predominantly up or down in the stem but amino acids and amides were also shown to move across the stem into the opposite primary leaf (19). The technique of labelling the products of photosynthesis starting with $^{14}CO_2$ or $^{13}CO_2$ has been used to study the translocation of sugars (25) (28) (17). The distribution of ^{14}C in the sugars of the fed leaf has been described for soybean (28). The ^{14}C incorporated into the sugars and amino acids by short periods of photosynthesis is characterized by about 60% of the ^{14}C in sucrose, small but equal amounts, around 8%, of ^{14}C in each of glucose and fructose, and the remaining ^{14}C is distributed in the amino acids such as α-alanine, glutamic acid, aspartic acid, glycine and asparagine. Of these compounds produced by photosynthesis, ^{14}C-sucrose is the main sugar translocated in soybean. Under certain conditions small amounts of serine and glycine are translocated as well (16).

The translocation of ^{3}H is not as extensively documented as the translocation of ^{14}C. Two groups have studied ^{3}H with conflicting results. Biddulph and Cory (2) found translocation of $^{3}H_2O$ comparable in amount to the translocation of ^{14}C-organic compounds. Gage and Aronoff (5) found no translocation of $^{3}H_2O$ as such and translocation of ^{3}H-organic compounds comparable in amount to that of ^{14}C-organic compounds. Neither group has studied which organic compounds are labelled by ^{3}H, either in the fed leaf or in the translocation stream.

Biddulph and Cory applied ^{3}H, ^{14}C and ^{32}P to the first trifoliate leaf of red kidney beans grown hydroponically and the individual isotopes were determined in successive stem sections approaching the roots. $^{3}H_2O$ and $Na^{32}PO_4$ were sprayed on the lower surface of the leaf while $^{14}CO_2$ was fed to the upper surface. The $^{3}H_2O$ was removed from the sections by freeze drying and the sections were then extracted with 80% ethanol to remove ^{14}C and ^{32}P. The $^{3}H_2O$ was reduced to $^{3}H_2$ and counted in the gas phase across a nichrome wire with a counting efficiency of 7%. The ^{14}C and ^{32}P, in a solid phase, were counted together in a windowless geiger counter. An aluminum filter separated the lower energy β particles of ^{14}C from the higher energy β particles of ^{32}P. The counting efficiency was 30% for ^{14}C and 85% for ^{32}P. The number of moles of ^{3}H, ^{14}C and ^{32}P per section was plotted against the position of

the section down the stem and all three isotopes decreased logarithmically down the stem. The fed leaf for a 30 minute feeding was given 2,500 μc of ^{3}H, 100 μc of ^{14}C and 160 μc of ^{32}P. Of these amounts only 2.5 mμc of ^{3}H, 5 mμc of ^{14}C and 3 mμc of ^{32}P were translocated. Thus the fraction translocated of the ^{14}C fed was fifty times the fraction translocated of the ^{3}H fed. The actual amount of water and organic compounds represented by the isotopes cannot be determined from the data given. Biddulph and Cory did not determine the amount of isotope present in the fed leaf nor the specific activity of the isotope in the fed leaf or in the translocation stream. The amount of isotope in the fed leaf would indicate how much of the isotope fed was actually taken into the leaf and the specific activity of the isotope in the translocation stream would indicate the amount of non-labelled compound that accompanied the labelled compounds. Unfortunately, it is impossible to measure the specific activity of the compounds in the translocating vessels without dilution by compounds in surrounding tissues.

The relative velocity of each isotope was calculated from the distance the front had moved since the beginning of feeding. Since isotopes present in large amounts are easier to detect than isotopes present in small quantities it is meaningless to compare the different fronts of isotopes of different specific

activity. Close to the front of movement less radioactivity is found because the radioactivity has a shorter time to accumulate and at the very front of movement radioactivity is difficult to detect. With low specific activity relatively little radioactivity is present and the front is hard to determine. Time zero for translocation velocities is not the time of entry into the leaf but the time of entry into the translocation stream. Time zero for each of the three isotopes is impossible to determine because of differences in penetration time. The ^{14}C goes through the process of photosynthesis and the $^{3}H_2O$ and ^{32}P penetrate from the lower outside surface of the leaf to the translocation stream before the beginning of translocation (29) (14). The lag times for $^{3}H_2O$ and ^{32}P have not been studied.

Gage and Aronoff (5) used soybean plants grown in soil in a greenhouse. The plants were watered 10 to 15 minutes before feeding in an attempt to eliminate internal water tension. The first trifoliate leaf was surrounded with $^{3}H_2O$ vapour of a specific activity of 10 mc per ml. The plant was allowed to carry on photosynthesis and translocation for 30 minutes before being cut in sections. Gage and Aronoff avoid the difficulty of differences in specific activity by labelling the water stream and the organic compounds with the same isotope from the same source. Thus ^{3}H fed as water vapour labelled the liquid water in the fed leaf and then labelled the sucrose formed in photosynthesis.

Water was removed from each section by freeze drying and organic compounds were extracted with hot ethanol. The ethanol-soluble fractions and the water fractions were counted separately using the technique of exchanging ^3H with hydrogen in NH_4Cl and counting the latter in the solid phase (10). The counting efficiency was 5%. The fed leaf contained 689,000 cpm of ^3H in ethanol-soluble organic compounds and 398,000 cpm of ^3H in water. The other parts of the soybean contained 36,620 cpm of ^3H in the ethanol-soluble organic compounds and 301 cpm of ^3H in water. Gagé and Aronoff assumed from analogy with $^{14}CO_2$ feedings that ^{14}C-sucrose was the main organic compound translocated but they were unable to test this hypothesis. About 5% of the ^3H in the organic compounds and about 0.1% of the ^3H present in the water of the fed leaf were translocated. Some of the ^3H_2O translocated into the stem may be converted there to organic compounds by photosynthesis making the ^3H_2O appear low. However, the translocation occurred to stems and root tips, areas which have only a limited amount of photosynthesis.

Although Biddulph and Cory, and Gage and Aronoff were able to demonstrate limited translocation of ^3H, they were hampered by the difficulties involved in counting the low energy β particles of ^3H. Sample preparation is extremely complicated when using the Geiger counter and the best counting efficiency achieved for

^{3}H was 7%. Their conflicting results, reflect this difficulty. Liquid scintillation assay with its simple methods for sample preparation and with a counting efficiency for ^{3}H of 30% introduces a more accurate technique for counting tritium. A re-examination of the movement of ^{3}H compounds and a comparison with the movement of ^{14}C could be profitable using liquid scintillation techniques.

For a sound basis of comparison of ^{14}C and ^{3}H translocated in different plants, the plants must be similar in their genetic composition, environmental conditioning and physiological development. Since workers in the field of agricultural research have sought a genetically homogeneous strain of soybean this is the best choice for translocation studies from the point of view of consistent genetic properties. Certified seed of the Comet or Hawkeye varieties can be obtained and has been used by Canadian workers (18) (19) (20) and Aronoff at Illinois (5). Plants grown from such seed are used with some success in translocation. However, it must be remembered that these plants are bred mainly for disease resistance and secondly for their uniform response to environment, in order to produce a high yield. These plants have a fairly uniform response to translocation. Examination of short-term translocation in hundreds of plants in both the N.R.C. and Queen's laboratories gives the following results. From a

heterozygous population of seeds translocation in a fifteen-minute experiment appears in only two out of each ten plants. With a more homozygous population of a variety such as Comet or Hawkeye, translocation is obtained in six out of ten plants. These differences among homozygous plants are probably due to the different responses of the plants to environment. As environmental control facilities improve and as our understanding of the environmental factors playing a part in translocation increase we can now obtain translocation in eight out of ten plants. However, the amount of material translocated in a thirty-minute experiment varies as much as a hundred times in different plants.

In all of the studies published to date some of these factors were controlled while others were not. It therefore seemed appropriate to make a careful study of translocation in which as many factors as possible were taken into account. The purpose of this study was to develop a "standard plant." This means a plant that will behave in a predictable way in experiments where translocation of ^{14}C and ^{3}H compounds are studied.

Genetic effects can be illustrated by a comparison of Comet and Hawkeye varieties. These varieties differ in sucrose content (20) (28). As sucrose is the main sugar translocated in soybean, differences in sucrose content could account for differences in the translocation rates reported by various workers in the literature.

Environmental effects can be demonstrated by comparing plants grown under various conditions. Plants grown in three different ways, hydroponically in a growth chamber, in vermiculite and tap water in a growth chamber, and hydroponically in a greenhouse supplemented with incandescent light show differences in external appearance, relative distance between internodes, thickening of stem and size of leaf (21). These plants translocated different percentages of sucrose, serine, and malic acid. Thus environment can promote variations in plants even though they are genetically similar. Environmental factors include temperature, light, water supply, mineral nutrition, concentration of O_2 and CO_2 and diurnal and seasonal variations that result in cyclic changes. These environmental factors will be discussed in relation to soybean plants.

Temperature has a wide effect on the growth of soybeans. Soybeans planted early in the spring take longer to germinate and the size of the primary leaf is small if temperature is low during early growth (3). A rise in temperature increases the rate of photosynthesis (1) and the rate of translocation (9). Clearly, the effect of temperature on growth should be taken into account when plants are compared for photosynthesis or translocation as the anatomy of the plant and the physiological processes are altered by changes in temperature. Optimal temperature conditions for growth should be established and this temperature regime kept constant.

Light can be divided into three factors for consideration - quality, quantity and photoperiod. Incandescent light must be added to fluorescent light to ensure good growth under artificial light conditions. The emission peaks of fluorescent light do not coincide with the chlorophyll absorption bands and must be supplemented with incandescent light which is rich in the red region. Soybean plants were taller and had lower dry weights when grown under incandescent light as compared to fluorescent light supplemented with illumination from a carbon arc lamp (23). Light intensity has a definite effect on plant growth. Most plants become etiolated under weak light and certain minimum light is necessary to obtain peak photosynthetic efficiency. The promotion of internode elongation by far red and reversal by red light has been shown for pinto beans (7). Photoperiod affects flowering in long and short day plants. Flowering in Biloxi soybean is effected by even a short period of light during the long dark period necessary to promote flowering (24). Since light quality, quantity and photoperiod can considerably affect the development of a plant, the light regime must be controlled to develop a standard plant.

Because soil acts as a reservoir for water, plants grown under natural conditions are not greatly affected over a wide range in water content of the soil. Plants grown in artificial conditions are not limited by ground water if care

is taken to neither over-water or under-water them. However, plants grown under artificial conditions are probably more susceptible to high humidity effects due to confinement. As humidity increases the diffusion gradient decreases and transpiration decreases. The absorption of water by transpiring plants is controlled largely by the rate of transpiration (11). Plants grown in high humidity tend to grow faster and develop thin walled succulent tissue. Plants at low humidity, with a marked internal water deficiency, grow slowly, develop thick walled cells and in general lack succulence (31). Extremes of humidity should be avoided, although plants will grow quite well over a wide range of intermediate humidity conditions. Growth cabinets, especially those with re-circulating air, can develop very high or very low humidities, making humidity control necessary.

Many mineral elements are essential for growth. Soybeans grown on a nitrogen free medium die after approximately 35 days. Mineral nutritional requirements for many plants have been determined for hydroponic growth. For example, Donovan's solution (19) has been developed specifically for soybean culture. Plants grow well in soil over a wide range of mineral concentrations due to the binding of ions to the soil particles. Ions are absorbed from the soil as required and toxic effects from high concentration of certain elements are eliminated by having the ions bound to the soil particles. Soils can vary

in their mineral content sufficiently to affect the growth of a standard plant. Therefore, soil is a poor medium for growing a standard plant, since if conditions are to be exactly duplicated at some later date the soil available may not be the same as in the original experiment. Conversely, in water culture the mineral requirements can always be duplicated. The determination of the mineral balance is essential for a standard plant since mineral deficiencies are difficult to diagnose before irreversible damage has been done.

The concentrations of CO_2 and O_2 affect the rate of photosynthesis and respiration. Under natural conditions no marked change occurs in the concentration of these two gases in the atmosphere. Under artificial growth conditions the air is continually being mixed and exchanged by air circulation and gas diffusion so CO_2 and O_2 concentrations never fluctuate enough to affect growth. However, oxygen supply to the roots is important and lack of oxygen has been shown to reduce top growth as much or more than root growth (8). This is an important factor for plants grown hydroponically and the culture solution must be aerated.

Flowering in plants is accompanied by large changes in physiological activity. Thus, the physiological stage of development is important in comparing different plants and environmental effects which could alter the transition between

different stages should be kept standard. Soybeans are also known to have daily rhythms which are related to daily environmental changes. The peak time for translocation occurs before ten o'clock in the morning. A second, smaller peak occurs in the late afternoon. A change in the light schedule affects the timing of these rhythms.

Physiological activity also changes with age. Labelled amino acids and amides introduced through the cut petiole of one primary leaf appear in the opposite primary leaf in different patterns depending on the age of the plant (20). The amount of serine translocated to the opposite leaf decreased with age while the amounts of asparagine and glutamine increased with age. This indicates that although the conductive elements were present and functional in the plants at all stages of development the destination of the amino acid or amide was controlled selectively. Thus in comparative studies in translocation it is important to select plants at the same stage of development.

In discussing the standard plant, confusion arises between a standard plant and a typical plant, i.e. the average plant present under normal field conditions. The standard plant is one which can be easily reproduced under defined conditions. For the sake of practical application the standard plant should be as close as possible to the typical plant but reproduction

of a given plant should not be sacrificed to obtain one which is more typical of field conditions. Once a physiological process is understood then this understanding of the process can be modified to meet changes found under natural conditions.

Due to the controversy concerning translocation of ^{3}H and the need for a "standard plant" to compare translocation studies, the aim of this thesis is threefold; first, to compare plants of various ages to find the best age of plant for short term translocation experiments, second, to develop methods necessary to assay ^{3}H in plant extracts and on paper chromatograms and third, to compare the ^{14}C-labelled products of photosynthesis and translocation with those labelled with ^{3}H.

Materials and Methods

Glycine max L. variety Comet was grown in a constant environment in hydroponic solution or in pots containing vermiculite. Illumination was 16-hours per day at a light intensity of 2400 ft-c. Plants at various stages of development were used for the experiments as follows:

Stage 0: Plants grown in hydroponic solution had fully expanded primary leaves.

Stage I: Plants grown in hydroponic solution or grown in vermiculite had one fully expanded trifoliate leaf.

Stage II: Plants grown in hydroponic solution had two fully expanded trifoliate leaves.

The hydroponic solution was modified Donovan's solution (19). This modified solution contained the same concentration of salts as previously published except Ca $(NO_3)_2.H_2O$ was half as concentrated (0.88 mmoles per litre) and $MgSO_4.7H_2O$ was three times as concentrated (3.46 mmoles per litre). P. R. Gorham, of the National Research Council, Ottawa, recommended the modified solution for the Comet variety of soybean. The 1000 ml. polyethylene culture flasks were sprayed on the outside with a layer of opaque black paint covered with aluminum. The plywood covers had one hole bored for the stem of the plant and one for an aeration tube. The plants were held

in place with a strip of sponge rubber. The beakers were aerated with a steady stream of air from glass tubing with a 1 mm. diameter orifice. Seedlings were sprouted in vermiculite in the growth chamber. After the fifth day, from the time of planting, soybeans of uniform size between 12 and 15 cm. from root tip to cotyledon node were placed in culture flasks. The plants were returned to the growth chamber until they were 14, 19 or 24-days-old. The roots were aerated continuously and the growth solution was changed every second day.

Plants grown on vermiculite were given a fertilizer supplement (HiSol 20-20-20) every third day starting on the fourteenth day.

The area of each leaf was determined by measuring a tracing with a planimeter.

Radioactive carbon in the form of sodium bicarbonate at a specific activity of 31.5 millicuries per gram was obtained from Atomic Energy of Canada Ltd. Tritiated water at a specific activity of 100 millicuries per gram was obtained from New England Nuclear Corporation.

To measure the apparent rate of photosynthesis, a primary leaf was enclosed in a plexiglass chamber with a total internal volume of 39 ml. Air was pumped from the leaf through an infra-red gas analyzer in a closed circuit and the change of CO_2 concentration with time was recorded (12). Light intensity

was 2000 ft-c at the surface of the primary leaf. The chamber was flushed with air and the apparent rate of photosynthesis measured three times. An average of the three values for change in CO_2 and the fresh weight of the primary leaf was used to calculate the apparent rate of photosynthesis in g $min^{-1}g^{-1}$ fr wt of net CO_2 uptake in the closed system. The variation between the three readings was less than 5%.

The translocation of the radioactive isotopes from a primary leaf to the other sections of the plant was measured in a fume hood under the same light conditions used to measure the apparent rate of photosynthesis. The plants were given a half hour pretreatment before feeding either ^{14}C or ^{3}H.

Each plant was fed 47.5 μc of ^{14}C. The ^{14}C was obtained as sodium bicarbonate and converted to carbon dioxide. The gas mixture was circulated around the primary leaf. The closed circuit also contained a geiger tube to measure the uptake of $^{14}CO_2$ and a 2000 ml reservoir to ensure that the carbon dioxide concentration did not fall to the carbon dioxide compensation point during the time of feeding.

Tritiated water (2500 μc) was fed to the primary leaf of each plant from a small chamber consisting of an open glass cylinder, 1.2 cm in diameter, with a side arm for holding liquid $^{3}H_2O$. The top was sealed with a rubber diaphram and the bottom sealed to the leaf with Apiezon stop-cock grease. The $^{3}H_2O$ in the side arm was converted to water vapour by heating the side arm.

At the end of the thirty-minute feeding the plant was cut into sections, the sections weighed, and extracted with 80% hot ethanol for ten minutes, followed by washing with hot ethanol. Both ethanol extracts were combined and evaporated to dryness on a steam bath under a steady flow of air blown into the evaporating extract. The extracts were taken up in a known volume of 80% ethanol. The extract from the fed leaf in the ^{14}C experiments was taken up in 5 ml. and all the other extracts were taken up in 1 ml. of alcohol.

The ^{14}C extracts were counted with a methane-flow proportional counter. A 50 µl aliquot of each extract was plated on an aluminum planchet. Duplicate planchets were made. Each planchet was counted for 10 minutes or until 10,000 counts were accumulated. The values were averaged and corrected for background. Duplicate planchets differed by less than 3%.

The ^{3}H extracts were counted with a liquid-scintillation spectrometer, Packard Tri-Carb model 3003. A 50 µl aliquot of each extract was dissolved in a vial containing scintillation fluid consisting of 5.0 g PPO[1], 0.3 g dimethyl POPOP[2] in 300 ml. dioxane made up to one litre with toluene. Duplicate vials were counted until they differed by less than 5%. In order to correct for quench and ^{14}C contamination and to convert from cpm to dpm the vials

---

[1] PPO - 2, 5 - diphenyloxazole

[2] dimethyl POPOP - 1, 4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene

were spiked with ^{3}H standard containing 1,998 dpm, recounted and then spiked with ^{14}C standard containing 438 dpm and recounted. The difference between the actual count rate for spiked and unspiked samples over the absolute radioactivity of the spike gives the counting efficiency for each isotope. Counting efficiency depends on the amount of quench and the width of the window on the "red" or "green" channel of the spectrometer. The "red" channel was set to count ^{3}H with a high efficiency and the "green" channel to count ^{14}C with a high efficiency. The radioactivity in dpm for both ^{3}H and ^{14}C for each sample was calculated from the efficiency of ^{3}H and ^{14}C counts in each channel and the unspiked count rate for the sample from each channel. The technique is illustrated in Tables 1 and 2. Table 1 gives the count rates spiked and unspiked and the absolute activities of the plant sections from soybean plant number 24 fed with $^{3}H_2O$. Table 2 gives a sample calculation for an aliquot taken from the fed leaf of this plant.

The ^{3}H products of photosynthesis were separated by two dimensional paper chromatography (26). The position of ^{3}H labelled compounds cannot be identified by autoradiography as the ^{3}H β-particle is not energetic enough. For each extract, replicate chromatograms were tested for sugars and amino acids. The sugar test was 0.5 ml. benzidine, 10 ml. acetic acid, 10 ml. 40% (w/v) trichloroacetic acid, and 80 ml. of 90% ethanol sprayed on the chromatograms and heated at 100-110°C for 5 to 10 minutes.

Table 1. Activity of aliquots of ethanol-soluble ^{3}H from sections of soybean plant fed with $^{3}H_2O$ (5.5×10^{10} dpm).

Plant Section	Aliquot Number	Unspiked Red (cpm)	Unspiked Green (cpm)	Spiked ^{3}H Red (cpm)	Spiked ^{3}H Green (cpm)	Spiked ^{14}C Red (cpm)	Spiked ^{14}C Green (cpm)	Absolute Activity ^{3}H (dpm)	Absolute Activity ^{14}C (dpm)
	background	28.1	22.6	22.6	26.7	20.9	25.3	-	-
fed leaf	.1	43.1	10.2	266.1	56.2	516.4	336.0	373	2.5
" "	.2	38.7	8.2	267.2	56.3	519.4	338.9	378	0.1
opposite primary leaf	.1	223.4	247.4	454.6	292.9	715.4	676.8	571	264
" "	.2	247.8	271.8	485.9	286.5	731.6	594.9	516	332
growing tip	.1	4.7	3.1	297.2	78.4	523.3	380.2	20	3.4
" "	.2	6.5	5.4	306.2	81.2	531.8	391.5	21	6.4

All values corrected for background except the background

Red Channel Gain 45% A^{50} to B^{1000}

Green Channel Gain 7.7% A^{50} to B^{1000}

Spikes ^{3}H 1,998 dpm

^{14}C 438 dpm

Table 2. Sample calculations for an aliquot taken from the fed leaf of plant 24.

Red Channel

$$^{3}H \text{ efficiency} = \frac{266.1 - 43.1}{1,998} = \frac{223}{1,998} = .1116$$

$$^{14}C \text{ efficiency} = \frac{516.4 - 266.1}{438} = \frac{250.3}{438} = .5715$$

Green Channel

$$^{3}H \text{ efficiency} = \frac{56.2 - 10.2}{1,998} = \frac{46.0}{1,998} = .02302$$

$$^{14}C \text{ efficiency} = \frac{336.0 - 56.2}{438} = \frac{279.8}{438} = .6388$$

Let t be the initial, unspiked, absolute count rate in dpm of ^{3}H

Let c be the initial, unspiked, absolute count rate in dpm of ^{14}C

Then $.1116t + .5715c = 43.1$ cpm (a)

$.02302t + .6388c = 10.2$ cpm (b)

Solving for t = 373 dpm

In (b) $.02302 \times 373 + .6388c = 10.2$

$c = 2.52$ dpm

In (a) $.1116 \times 373 + .572 \times 2.52 = 41.66 + 1.44$

$= 43.1$

The amino acids were located with ninhydrin (27). Spots corresponding to the positions of the sugars or amino acids were cut from a third unsprayed chromatogram. These spots were placed directly in vials containing the same scintillator fluid without the dioxane, as used to count the ethanol aliquots. The counting of the spots gave the relative activity of each compound found on the chromatograms. The background was taken from a vial containing a piece of non-radioactive paper which had been run through the chromatogram solutions.

The geometry of the chromatogram spots within the vial was tested to determine if the orientation of the spot had an effect on the count rate. ^{14}C-glucose was spread evenly over pieces of chromatogram paper 2.56 cm^2 in area. The paper was oriented in the vials in the following ways:

1. A square (1.6 x 1.6 cm) lying flat on the bottom of the vial.
2. A square (1.6 x 1.6 cm) folded diagonally so half was flat on the bottom and half standing vertically in the vial.
3. A rectangle (5.0 x 0.51 cm) wrapped around the inside of the vial about half way from the bottom.
4. A rectangle (2.5 x 1.02 cm) folded longitudinally resting on the bottom of the vial.

5. A rectangle (2.5 x 1.02 cm) folded longitudinally and standing upright in the vial.

The Student-Fisher-t test showed no significant difference at the 5% level in the count rate between the various orientations, Table 3.

Paper squares (1.6 x 1.6 cm) orientated with the pipetted side either up or down showed no significant difference at the 5% level in the count rate in these two orientations, Table 4.

Thus for ^{14}C the geometry of the chromatogram spot has no effect on the count rate. However, a discrepancy due to orientation of "not more than 10%" has been reported for counting tritium spots (4). For this reason, the method of counting spots containing ^{3}H was checked with the results shown in Table 5. The spot was oriented in the vial in three positions:

1. Suspended half way from the bottom of the vial normal to the receiving surface of the photomultiplier.
2. Suspended half way from the bottom of the vial perpendicular to the receiving surface of the photomultiplier.
3. Flat on the bottom of the vial.

Table 3. Count rates for five orientations of paper strips spread evenly with 25 µl ^{14}C glucose 500 cpm/25 µl.

Orientation of strip	count rate cpm	mean	s	t[1]
Square (1.6 x 1.6 cm) flat on bottom	481.7 333.6 405.5	406.9	60.7	.168
Square (1.6 x 1.6 cm) folded diagonally	414.9 413.9 415.8	414.9	.77	.237
Rectangle (5.0 x 0.51 cm) wrapped around vial	421.1 408.9 419.8	416.6	1.39	.338
Rectangle (2.5 x 1.02 cm) on bottom	415.5 415.5	415.5	0	.421
Rectangle (2.5 x 1.02 cm) vertical	406.6 390.5 410.1	402.4	8.53	.512

[1] Student-Fisher-t for comparing the differences between two sample means when the population variances are unknown (13). Each sample mean was compared with a mean of all sample values (mean 411.0, s = 27.2).

Conclude: Student-Fisher-t for all samples was not large enough to indicate a significant difference at the 5% level between the sample means and a total mean.

Table 4. Count rates of two orientations of pipetted side of paper strip (1.6 x 1.5 cm) spread evenly with 25 µl ^{14}C glucose 600 cpm/25 µl.

Orientation of strip	count rate cpm	mean	s	t[1]
Square (1.6 x 1.6 cm) pipetted side up	130.9 149.4 143.3	141.2	7.7	.44
Square (1.6 x 1.6 cm) pipetted side down	139.1 141.9 145.0	142.0	2.4	

[1] Student-Fisher-t for comparing the differences between two sample means when the population variances are unknown (13). Each sample mean was compared with a mean of all sample values (mean 411.0, s = 27.2).

Conclude: Student-Fisher-t was not large enough to indicate a significant difference at the 5% level between the two sample means.

Three replicate counts were made of the same spot in the three different positions. A consistent position to the photomultiplier could not be achieved in the automatic system because the vials turn on the track. Thus each vial was placed in the machine manually. The vial was recounted after the spot was removed to show that the geometry had not been upset by ^{3}H-glycine dissolving in the scintillation fluid. The spot flat on the bottom showed a 20% loss of cpm. Thus the orientation of the spot is important when ^{3}H is assayed. The easiest geometry to reproduce in the automatic counting system is flat on the bottom of the vial and this orientation was used throughout.

Table 5. Count rates for three orientations of paper spot with ^{3}H-glycine.

Orientation of spot	count rate cpm	mean	s
Vial without spot background	11.1 14.0 11.7	12.3	1.2
Spot suspended in vial normal to the receiving surface of the photomultiplier	105.6 101.0 99.7	102.1	2.9
Spot suspended in vial perpendicular to the receiving surface of the photomultiplier	113.0 111.2 105.6	109.9	3.2
Spot flat on bottom of vial	84.7 84.7 83.2	84.2	.7
Vial after spot removed	12.2 11.3 12.9	12.1	.6

Results

I Development of Young Soybean Plants

Chronological age is not a satisfactory indication of development in bean plants. There is a regular progression from germination of the seed, to development of the primary leaves and to the expansion of the trifoliate leaves. It is reasonable to break this pattern of development into regular stages. Thus, the plant with primary leaves fully expanded can be recognized as one stage of development, called Stage 0, the plant with primary leaves and one trifoliate leaf fully expanded, Stage I, and the plant with primary leaves and two trifoliate leaves fully expanded, Stage II. Under controlled culture conditions in a growth chamber the primary leaves are fully developed from 12 to 15 days after germination of the seed. There is a 3 to 5 day interval between the development of each of the succeeding trifoliate leaves.

Plants grown hydroponically for 14, 19 and 24-days were Stage 0, Stage I and Stage II plants. The variation in the weights of various plant parts and leaf areas were compared to obtain a record of each age group of plants to be used as experimental material. Weights of plants grown in vermiculite with added nutrient for 24 days were compared with weights of plants grown hydroponically for 19 days. These two groups of plants were at the same stage of development, namely, Stage I.

The weights of plant parts and leaf areas of 14-day-old plants (Stage 0) are shown in Table 6. The stem below the

Table 6. Weight and leaf area of sections of 14-day-old plants (Stage 0).

Plant Part	Plant 4 g (cm^2)	5 g (cm^2)	6 g (cm^2)	7 g (cm^2)	8 g (cm^2)	9 g (cm^2)	14 g (cm^2)	22 g (cm^2)	23 g (cm^2)	mean	s	variation[1]
First trifoliate leaf	.12	.16	.11	.10	.18	.26	.03	.08	.14	.13	.07	54
Fed primary leaf	.34 (25.8)	.35 (24.9)	.33 (24.8)	.25 (17.1)	.31 (25.8)	.32 (28.5)	.13 (13.4)	.23 (21.4)	.30 (18.3)	.28 (22.2)	.08 (4.8)	29 (20)
Opposite primary leaf	.43 (27.5)	.38 (27.7)	.42 (26.1)	.24 (19.9)	.30 (25.6)	.32 (26.3)	.12 (11.4)	.22 (21.1)	.30 (19.5)	.30 (22.8)	.10 (5.0)	33 (20)
Stem below primary leaves	1.42	1.38	1.56	1.53	1.51	1.56	1.36	1.40	1.41	1.46	.05	3
Root	.96	1.01	1.00	.75	.94	.95	.62	.90	.97	.90	1.25	14

1 Variation is the standard deviations expressed as per cent of the mean.

primary leaf made up about half the total weight of the plant. The roots and primary leaves accounted for almost all of the rest. The trifoliate leaf was just beginning to develop in plants of this age. The variation in the weights of the different plant parts was surprisingly small. For example, the smallest variation, $\pm 3\%$,[1] occurred in the stem below the primary leaf while the largest variation, $\pm 54\%$, occurred in the emerging first trifoliate leaf. This first trifoliate leaf was a rapidly-growing shoot and was expected to vary greatly in weight. The variation in weight of the other plant parts fell between these two values.

The leaf areas for the fed primary leaf and opposite primary leaf were 22.8 ± 5.0 cm^2 and 22.2 ± 4.8 cm^2. This is a variation of 20% for both values. Although leaf areas were more consistent measurements than the weights, it makes little difference in the experiments described below which is taken as an index of development of the leaves.

The weights of plant parts and leaf areas of 19-day-old plants (Stage I) are shown in Table 7. The stem and the root were about equal in weight and made up the bulk of the plant body. The first trifoliate leaf was about twice as big as a primary leaf when expressed either as fresh weight or as

---

[1] Standard deviations are expressed as percent of the mean.

Table 7. Weight and leaf area of sections of 19-day-old plants (Stage I).

Plant Part	Plant 10 g (cm^2)	11 g (cm^2)	12 g (cm^2)	13 g (cm^2)	19 g (cm^2)	mean	s	variation [1] %
Third trifoliate leaf	0.06	0.06	0.03	0.02	0.05	.04	.02	50
Second trifoliate leaf	0.15 (9.9)	0.12 (5.0)	0.09 (4.2)	0.04 (1.6)	0.14 (11.0)	.11 (6.3)	.04 (3.5)	36 (56)
Stem between first and second trifoliate leaves	0.25	0.18	0.16	0.07	0.19	.17	.12	70
First trifoliate leaf	0.54 (56.5)	0.58 (42.1)	0.84 (42.5)	0.34 (34.4)	0.50 (55.1)	.56 (46.1)	.16 (8.4)	29 (18)
Stem between primary and first trifoliate leaves	0.42	0.83	0.28	0.23	0.25	.40	.23	58
Fed primary leaf	0.30 (28.6)	0.27 (25.2)	0.32 (32.0)	0.25 (26.9)	0.19 (20.9)	.27 (26.7)	.04 (3.67)	15 (14)
Opposite primary leaf	0.33 (30.2)	0.29 (29.4)	0.29 (26.3)	0.24 (25.1)	0.24 (22.2)	.28 (26.7)	.05 (2.9)	18 (11)
Stem below primary leaves	1.51	1.63	1.66	1.14	1.18	1.42	.25	18
Root	1.53	1.49	2.11	1.38	1.61	1.62	.28	17

1 Variation is the standard deviations expressed as a per cent of the mean.

leaf area. The second trifoliate was small and not fully expanded. The variations in the weights of the plant parts were similar to those observed for the younger Stage 0 plants and were from ± 17% to ± 18% for the primary leaves and from ± 29% to ± 70% for the rapidly growing trifoliate leaves and stem parts above the primary leaf.

Using the weights of the various plant parts as an index of development the 14-day-old and 19-day-old plants were considered consistent enough material to be used in translocation experiments.

The weights of plant parts and leaf areas of 24-day-old plants (Stage II) are shown in Table 8. The stem below the primary leaf and the primary leaves of the Stage II plant did not increase in weight from the Stage 0 or Stage I plants. The first and second trifoliate leaves were three times as large as the primary leaves in weight and leaf area. The third trifoliate leaf was slightly larger than the primary leaves in weight and leaf area but did not reach the full size of the first and second trifoliate leaves. The fourth trifoliate leaf was just emerging. The plant body above the primary leaves was about the same weight as the roots and each accounted for well over one-third of the weight of the plant. The variations in weight of the plant parts below the primary leaf node were the same as the variation found for the same parts in the Stage 0 and Stage I

Plant Part	2 g (cm^2)	3 g (cm^2)	15 g (cm^2)	16 g (cm^2)	17 g (cm^2)	mean	s	variation[1] %
Fourth trifoliate leaf	.28	.25	.03	0.03	0.08	.13	.10	85
Third trifoliate leaf	.51 (67.7)	1.22 (91.0)	.06 (2.1)	0.07 (1.4)	0.19 (15.8)	.41 (35.6)	.44 (38.0)	107 (107)
Stem between second and third trifoliate leaves	.32	.24	.35	0.49	0.36	.35	.13	37
Second trifoliate leaf	1.45 (102.1)	1.29 (96.4)	.55 (53.5)	0.58 (58.9)	0.70 (61.2)	.91 (74.4)	.37 (22.4)	41 (30)
Stem between first and second trifoliate leaves	.22	.23	.40	0.43	0.35	.33	.07	21
First trifoliate leaf	1.32 (98.1)	1.09 (82.1)	.82 (78.2)	0.93 (80.6)	0.79 (76.3)	.99 (80.3)	.20 (22.6)	20 (28)
Stem between primary and first trifoliate leaves	.21	.26	.37	0.38	0.29	.30	.07	23
Fed primary leaf	.40 (32.6)	.47 (37.5)	.27 (24.4)	0.33 (31.0)	0.28 (25.9)	.36 (29.2)	.08 (9.1)	22 (27)
Opposite primary leaf	.31 (29.9)	.41 (32.8)	.30 (28.6)	0.35 (30.1)	0.27 (24.5)	.33 (29.2)	.11 (6.8)	33 (20)
Stem below primary leaves	1.83	1.65	1.44	1.45	1.12	1.50	.23	15
Root	3.04	3.24	4.18	4.23	2.43	3.42	.64	19

[1] Variation is the standard deviations expressed as a percent of the mean

plants. The weights of plant parts between the primary leaf node and the growing tip showed variations from ± 21% to ± 10%. In the 24-day-old plant large variations occur above the primary leaves which is the part of the plant making up 40% of the plant weight. The 24-day-old plants have grown too far beyond the primary leaf node to be considered consistent material for translocation experiments described below.

The weights of plant parts of 24-day-old plants (Stage I) grown in vermiculite with added nutrient are shown in Table 9. The roots accounted for half the weight of the plant and with the stem below the primary leaves accounted for the bulk of the plant weight. The first trifoliate leaf was the same weight as the primary leaves and had not reached full development. The second trifoliate leaf was just emerging. The primary leaves were the same weight as the Stage I plants grown hydroponically. The stem below the primary leaves was only half the weight of the plants grown hydroponically. The rest of the plant parts were within the range of the corresponding parts of the Stage I plants grown hydroponically. Plants grown in vermiculite showed greater variation than plants grown hydroponically. However, for the plants grown in vermiculite, the greatest variation occurred in the plant parts above the primary leaf node which accounted for less than one-fifth the plant weight. This was not considered a sufficient weight to make the variation

Table 9. Weight of sections of 24-day-old soybean plants grown in vermiculite (Stage I)

Plant Part	Plants 24 g	25 g	26 g	27 g	28 g	mean	s	variation[1] %
Second trifoliate leaf	.10	.10	.06	.72	.08	.21	.25	119
First trifoliate leaf	.40	.28	.18	.78	.19	.37	.22	79
Stem between primary and first trifoliate leaves	.16	.15	.21	.65	.10	.25	.21	84
Fed leaf	.32	.33	.23	.78	.24	.38	.20	53
Opposite primary leaf	.30	.33	.24	.76	.25	.37	.21	57
Stem below primary leaves	.55	.61	.53	.71	.51	.58	.08	14
Root	2.86	3.04	1.40	2.50	1.69	2.30	.67	34

[1] Variation is the standard deviation expressed as a percent of the mean.

important. Plants grown either in vermiculite of hydroponically up to Stage I were considered comparable plants for translocation experiments.

II Translocation and Distribution of Photosynthetically-Assimilated ^{14}C in Young Soybean Plants.

Soybean plants 14, 19 and 24-days-old, grown hydroponically, were chosen for experimental material. The rate of photosynthesis of one primary leaf of each plant was measured and expressed at $\mu g\ min^{-1}g^{-1}$fr wt. of net CO_2 uptake in the closed system. The total ^{14}C translocated throughout the plants was determined. In addition, the distribution of translocated ^{14}C among the various parts of the plants such as roots, leaves and internodes of the stem, was determined.

The rate of photosynthesis, the total ^{14}C in the ethanol soluble fraction of the fed leaf and the ^{14}C translocated in 14, 19 and 24-day-old plants are shown in Tables 10, 11 and 12 respectively.

The rate of photosynthesis of an attached 14-day-old soybean primary leaf was $155 \pm 18\ \mu g\ min^{-1}g^{-1}$fr wt (Table 10). The variation of 11.6% in rate of photosynthesis is reasonable for tissues of this kind, when the carbon dioxide analyzer is used. From the point of view of the rate of photosynthesis, 14-day-old soybean plants grown under these conditions can be considered "standard" plants.

Table 10. Assimilation and translocation of ^{14}C in 14-day-old soybean plants (Stage 0)
(47.9 μc $^{14}CO_2$ fed to one primary leaf for 30 minutes)

Plant	Rate of Photosynthesis μg $min^{-1}g^{-1}$fr. wt.	Total ^{14}C in ethanol-soluble fraction of fed leaf μc	Total ^{14}C translocated mμc
4	130	20.3	340
5	127	16.9	752
6	158	12.7	370
7	172	10.7	386
8	184	14.4	293
9	163	9.5	278
14	150	8.5	769
22	156	10.3	113
23	156	11.9	112
mean	155	12.8	379
s	18	3.6	220

The total ^{14}C in the ethanol-soluble fraction of the fed leaf of these same plants was 12.8 ± 3.6 μc, or about one-quarter of the total $^{14}CO_2$ fed to the primary leaf in 30 minutes. The variation of 21% was higher than the variation for the rate of photosynthesis but it is the variation expected in this kind of experiment. For example, about half of the ^{14}C offered was fixed in 30 minutes and of this 40 to 60% could be accounted for in the ethanol-soluble fraction. At least half of the ethanol-soluble ^{14}C is in the form of sucrose (20). The remainder is accounted for in the ethanol-insoluble fraction which is mainly starch (28).

The mean ^{14}C translocated was 379 ± 220 mμc. In 30 minutes essentially all of the translocated ^{14}C is in the form of sucrose (20). The variation in the amount of ^{14}C translocated is always large in an experiment as short as 30 minutes, since there is a 1 to 15 minute lag between the time when ^{14}C is first fixed in photosynthesis and the time the ^{14}C reaches the translocation stream (29) (14).

In translocation studies, it has been assumed that the amount of ^{14}C ethanol-soluble material (mainly sucrose) bears some relation to the ^{14}C translocated and also to the total ^{14}C fixed. However, variation among different plants has been so great that this assumption has not been tested.

Analysis of the data for 14-day-old plants is shown in the Appendix in Tables A 1, A 2, and A 3. At the 5% level of

significance there is no correlation between (a) the rate of photosynthesis and the total ^{14}C in the ethanol-soluble fraction of the fed leaf, (b) the total ^{14}C in the ethanol-soluble fraction of the fed leaf and the total ^{14}C translocated, and (c) the rate of photosynthesis of the fed leaf and the total ^{14}C translocated. Similar analysis for five 19-day-old plants, Tables 11, A 4, A 5, and A 6, shows no correlation at the 5% level between any of these three parameters. Such an analysis shows that neither the rate of photosynthesis nor the ^{14}C in the ethanol-soluble fraction of a fed primary leaf can be used as an index of translocation in young plants grown under standard conditions.

The results for five 24-day-old plants are shown in Table 12. Only three of these five plants translocated any ^{14}C and only two of these three translocated a significant amount. Since the 14 and 19-day-old plants were good material for translocation studies, further experiments with the older 24-day-old plants were discontinued.

All of the data for the 14, 19 and 24-day-old plants are summarized in Table 13. The rate of photosynthesis of one of the primary leaves showed little variation among each age group (Table 13). The rate of photosynthesis in the 14-day-old plants was significantly lower than the rate in the 19-day-old plants

Table 11. Assimilation and translocation of ^{14}C in 19-day-old soybean plants (Stage I) (47.9 μc $^{14}CO_2$, fed to one primary leaf for 30 minutes).

Plant	Rate of Photosynthesis	Total ^{14}C in ethanol-soluble fraction of fed leaf	Total ^{14}C Translocated
	μg $min^{-1}g^{-1}$ fr. wt.	μc	mμc
10	182	15.5	46
11	223	14.5	50
12	221	19.5	116
13	241	12.2	295
19	202	12.8	138
20[1]	99	9.5	39
21[1]	105	7.3	10
mean	214	14.9	129
s	18	2.6	90

1 Plants 20 and 21 had a serious fungus infection of the roots and are not included in the calculation of the mean and standard deviation.

Table 12. Assimilation and translocation of ^{14}C in 24-day-old soybean plants (Stage II) (47.9 μc $^{14}CO_2$ fed to one primary leaf for 30 minutes).

Plant	Rate of Photosynthesis	Total ^{14}C in ethanol-soluble fraction of fed leaf	Total ^{14}C Translocated
	μg $min^{-1}g^{-1}$ fr. wt.	μc	mμc
2	184	17.0	549
3	241	21.5	82
15	171	27.4	11
16	200	15.7	0
17	125	10.7	0
mean	184	18.5	213[1]
s	37.8	5.5	246

1 In calculating the mean and s for the total ^{14}C translocated plants 16 and 17 were disregarded as they did not translocate any ^{14}C in the 30 minute period.

Table 13. Summary of assimilation and translocation of ^{14}C in 14, 19 and 24-day-old soybean plants.

Age day	Rate of Photosynthesis $\mu g\ min^{-1} g^{-1}$ fr wt	Total ^{14}C in ethanol-soluble fraction of fed leaf μc	Total ^{14}C translocated $m\mu c$
14	155 $\pm$ 11.6% [1]	12.8 $\pm$ 20.8%	379 $\pm$ 58.0%
19	214 $\pm$ 8.4%	14.9 $\pm$ 17.5%	129 $\pm$ 69.8%
24	184 $\pm$ 20.6%	18.5 $\pm$ 20.6%	213 $\pm$ 115.5%

1 Variation is the standard deviation expressed as a percent of the mean.

(Table A 7), and in the 24-day-old plants (Table A 8). There was no significant difference in the rate of photosynthesis in the 19-day-old and 24-day-old plants (Table A 9). However, the 24-day-old plants showed a greater variation than the other two age groups. This variation undoubtedly reflects senescence of the primary leaf and was one of the reasons the older plants were not tested further.

The amount of ^{14}C in the ethanol-soluble fraction of the fed leaf was more variable than the rate of photosynthesis (Table 13). There was no significant difference between total ^{14}C in the ethanol-soluble fraction of the fed leaf in the 14 and 19-day-old plants (Table A 10). Both these values are significantly less than the ^{14}C in the ethanol-soluble fraction of the fed leaf of the 24-day-old plants (Tables A 11, A 12). This is probably due to the fact that bean leaves approaching senescence no longer synthesize starch.

The total ^{14}C translocated was more variable than either the rate of photosynthesis or the total ^{14}C found in the ethanol-soluble fraction of the fed leaf (Table 9). Even though these values are highly variable, they are less variable than translocation values previously recorded. Total ^{14}C translocated decreased in amount and became more variable with age.

The comparisons of the soybean plants of different ages indicated that the 14 and 19-day-old plants translocated

consistently. They were good material for translocation studies. The 24-day-old plants did not translocate consistently and were not suitable experimental material.

The distribution of translocated ^{14}C was determined by assaying the ethanol-soluble fraction of each plant part. It has been found that in a 30-minute experiment there was essentially no ^{14}C in the insoluble fractions of all parts other than the fed leaf. Therefore, the ethanol-soluble ^{14}C is a measure of the total translocated ^{14}C in each plant part. These parts were the stem beneath the fed primary leaf, the roots, the leaf opposite the fed primary leaf, and the parts of the plant above the fed primary leaf.

The 14-day-old plants had a very small emerging trifoliate leaf and stem above the fed primary leaf. The 19-day-old plants had both a first trifoliate, and a second trifoliate as well as an emerging third trifoliate leaf. The stems between the primary leaves and the first trifoliate leaf, and the first trifoliate leaf and the second trifoliate leaf were large enough to be extracted separately. In the 24-day-old plant the fourth trifoliate leaf had begun to emerge and the stem between the second and third trifoliate leaves was large enough to be extracted separately.

The ^{14}C translocated to the various parts of the 14, 19 and 24-day-old plants is expressed as percent of total activity translocated (Tables 14, 15, 16). The data are

Table 14. Distribution of translocated ^{14}C in a stage 0 soybean plant after 30 minutes: as a percent of total ^{14}C translocated.

Plant Part	Plant 4 %	5 %	6 %	7 %	8 %	9 %	14 %	22 %	23 %	mean	s
First trifoliate leaf	79.6	64.9	28.8	29.1	60.3	55.5	29.6	53.4	50.6	50.2	16.8
Opposite primary leaf	.2	.1	.2	.4	.8	.4	.0	1.0	0.0	0.3	0.4
Stem below primary leaves	19.8	34.6	58.3	56.7	35.2	38.0	68.1	45.6	48.5	45.0	14.1
Roots	.3	.4	12.7	13.8	3.7	6.1	2.3	0	.9	4.5	4.8

Table 15. Distribution of translocated ^{14}C in a stage I soybean plant after 30 minutes: as a percent of total ^{14}C translocated.

Plant Part	Plant						
	10 %	11 %	12 %	13 %	19 %	mean	s
Third trifoliate leaf	0.0	0.0	12.8	6.2	1.8		
Second trifoliate leaf	17.4	0.0	4.8	7.1	7.1		
Stem between first and second trifoliate leaves	0.0	61.5	11.9	7.0	9.3		
First trifoliate leaf	5.7	4.6	0.0	16.8	0.2		
Stem between primary and first trifoliate leaves	37.2	9.5	23.6	15.6	18.4		
Total up	60.3	75.6	73.1	52.7	36.8	59.7	14.4
Opposite primary leaf	7.3	7.9	9.3	1.7	0.0	5.2	8.2
Stem below primary leaves	32.4	16.5	36.4	39.2	45.8	34.1	9.7
Root	0.0	0.0	1.2	11.9	17.4	6.1	7.2

Table 16. Distribution of tranlocated ^{14}C in a stage II soybean plant after 30 minutes: As a percent of total ^{14}C translocated.

Plant Part	Plant 2 %	Plant 3 %	Plant 15 %	mean	s
Fourth trifoliate leaf	0.6	3.0	2.2		
Third trifoliate leaf	1.1	3.6	2.3		
Stem between third and second trifoliate leaves	0.0	0.0	0.0		
Second trifoliate leaf	0.0	1.2	2.4		
Stem between second and first trifoliate leaves	0.3	1.7	2.1		
First trifoliate leaf	0.2	1.7	1.5		
Stem between primary and first trifoliate leaves	15.7	25.5	4.1		
Total up	17.9	36.7	14.6	23.1	9.2
Opposite primary leaf	1.6	4.8	1.5	2.6	1.6
Stem below primary leaves	60.4	52.3	82.7	65.1	12.8
Root	20.1	6.2	1.2	9.1	8.0

Table 17. Summary of distribution of total ^{14}C translocated to various parts of the 14, 19 and 24-day-old plants.

Plant part	Age of Plant days		
	14 %	19 %	24 %
Parts above primary leaf	50.2 ± 16.8[1]	59.7 ± 14.4	23.1 ± 9.2
Opposite primary leaf	0.3 ± 0.4	5.2 ± 8.2	2.6 ± 1.6
Stem below primary leaves	45.0 ± 14.1	34.1 ± 9.7	65.1 ±12.8
Roots	4.5 ± 4.8	6.1 ± 7.2	9.1 ± 8.0

1. Standard deviations.

summarized in Table 17. In the 14 and 19-day-old plants approximately half of the ^{14}C translocated was found in the part of the plants above the fed leaf after a feeding of 30 minutes. In the 24-day-old plant only one quarter of the ^{14}C was found in the plant above the fed leaf. Most of the remaining ^{14}C was found in the stem below the primary leaf 45.0% ± 14.1, 34.1% ± 9.7 and 65.1% ± 12.8 for the 14, 19 and 24-day-old plants respectively. A small amount of ^{14}C was found in the opposite primary leaf and the roots in the three age groups. There is an indication that the pattern of translocation changed with age. The 14 and 19-day-old plants translocated relatively more material up from the fed leaf to the rapid growing shoot than the 24-day-old plants which exported relatively more material to the lower stem and roots. However, analysis of more 24-day-old plants needs to be made to be certain of the effect of age.

To summarize, in 30 minutes, C^{14} fixed in photosynthesis in the primary leaf was translocated mainly up and down the stem but not into the opposite primary leaf.

III Translocation and Distribution of Photosynthetically-Assimilated ^{3}H in Young Soybean Plants

Soybean plants grown in vermiculite with supplementary nutrition were chosen when they had one fully expanded trifoliate

leaf. These plants were comparable to the 19-day-old plants used in the ^{14}C experiments. Tritiated water was fed as water vapour to one primary leaf for 30 minutes. During extraction, all residual tritiated water was distilled from the extracts leaving tracer only in those organic compounds formed in photosynthesis. The total ^{3}H translocated throughout the plants was determined. Finally the weight of each plant part was determined.

The total ^{3}H in the ethanol-soluble fraction of the fed leaf and the total ^{3}H translocated are shown in Table 18. The total ^{3}H in the ethanol-soluble fraction of the fed leaf was 5.95 mμc $\pm$ 2.37 which is about 0.00024% of the total $^{3}H_2O$ fed to the primary leaf in 30 minutes. The uptake of ^{3}H cannot be measured with a geiger tube as can the uptake of ^{14}C so there is no indication of how much $^{3}H_2O$ was taken in by the fed leaf. Because all the liquid $^{3}H_2O$ in the side arm disappeared during the 30 minute feeding time it is reasonable to assume that considerably more $^{3}H_2O$ was taken up by the fed leaf than 0.00024% indicated by the ^{3}H present in the ethanol-soluble fraction of the fed leaf. This lost ^{3}H could be either in the form of $^{3}H_2O$ or ethanol-insoluble, ^{3}H-compounds. The ethanol-insoluble compounds were not counted and the $^{3}H_2O$ was lost in evaporating the ethanol extracts to dryness.

Table 18. Assimulation and translocation of ^{3}H in stage I soybean plants (2500μc $^{3}H_2O$ fed to one primary leaf for 30 minutes).

Plant	Total ^{3}H in ethanol-soluble fraction of fed leaf	Total ^{3}H translocated
	mμc	mμc
24	3.418	5.136
25	1.464	0.0
26	9.064	4.691
27	4.764	7.423
28	6.018	0.0
mean	5.95	5.75[1]
s	2.37	1.18

1. Mean and s based on three plants which translocated.

Only three out of the five plants tested had enough ^{3}H in the fed leaf to be detected by the methods developed in this thesis. The total ^{3}H translocated in the ethanol-soluble fraction was 5.75 mμc ± 1.18 taken as a mean of the three plants which translocated (Table 18). An equal amount of ^{3}H was found in the ethanol-soluble fraction of the fed leaf.

The distribution of translocated ^{3}H in the soybean plants showing ^{3}H translocation after 30 minutes is shown in Table 19. The roots received 29.9%, the stem below the primary leaf 12.1%, the opposite primary leaf 56.4% and the plant parts above the primary leaf 5.7% of the total ^{3}H translocated. The opposite primary leaf and root received the largest amount of ^{3}H translocated. When this distribution is compared to that obtained with ^{14}C as the tracer (Table 17), it must be concluded that the distribution patterns of ^{3}H and ^{14}C after 30 minutes translocation were entirely different.

IV The Distribution of ^{3}H in Organic Compounds of the Fed Primary and Opposite Primary Leaves

The translocation of ^{14}C in young soybean plants was mainly to the stem below the primary leaf and to the parts of the plant above the primary leaves (Table 17). The translocation of ^{3}H in the young soybean was mainly to the opposite primary

leaf and to the roots. The distribution of ^{14}C in the organic compounds of the fed leaf is known. It is also known that ^{14}C is mainly translocated as sucrose (28) (20). The distribution of ^{3}H in the organic compounds of the ethanol-soluble extract of the fed leaf and the form in which ^{3}H is translocated to other parts of the soybean has not been determined previously.

The ethanol-soluble extracts isolated from plants number 26 and 28 which were used for the distribution pattern of ^{3}H in the plant (Table 18) were also tested for the distribution of ^{3}H among the organic compounds. These extracts were separated into amino acids and sugars by paper chromatography and the chromatographic spots counted in the scintillation counter.

The distribution of ^{14}C and ^{3}H in the labelled compounds in the ethanol-soluble fraction of soybean leaves is compared in Table 20. The sucrose in the ethanol soluble fraction of the fed leaf contained 60% of the ^{14}C. Glucose and fructose both contained 8% of the total ^{14}C. The amino acids, α-alanine, glutamic acid, aspartic acid, glycine and asparagine, contained 22% of the total ^{14}C. The sucrose in the ethanol soluble fraction of the fed leaf contained about half the total ^{3}H. Again glucose and fructose both contained the same amount or $\pm$ 5% of the total ^{3}H. The amino acids, α-alanine, glutamic acid, aspartic acid, glycine and asparagine contained $\pm$ 35% to $\pm$ 48% of the total activity. The sucrose contained slightly less of the total ^{3}H and the amino acids slightly more of the total ^{3}H than was

Table 19. Distribution of translocated ^{3}H in a stage I soybean plant after 30 minutes: as a percent of total ^{3}H translocated.

Plant Part	Plant 24 %	26 %	27 %	mean	s
Second trifoliate leaf	4.0	0	0		
First trifoliate leaf	0	10.3	2.7		
Stem between primary and first trifoliate leaves	0	0	0		
Total up	4.0	10.3	2.7	5.7	3.1
Opposite primary leaf	96.0	61.1	12.1	56.4	34.4
Stem below primary leaves	0	24.2	12.1	12.1	36.2
Root	0	4.4	85.2	28.9	22.6

Table 20. Distribution of ^{14}C or ^{3}H in organic compounds of ethanol-soluble fraction of soybean leaves.

	% cpm of total cpm found in chromatogram				
	^{14}C [1] fed primary leaf	^{3}H fed primary leaf		^{3}H opposite primary leaf	
		Plant 26	Plant 28	Plant 26	Plant 28[2]
	%	%	%	%	%
sucrose	60	42	53	8	—
glucose	8	5	6	14	—
fructose	8	5	5	0	—
α-alanine	6	14	4	17	—
glutamic acid	4	13	19	21	—
aspartic acid	2	2	0	2	—
glycine	2	16	10	33	—
asparagine	1	3	4	5	—
others	12	—	—	—	—

1 The ^{14}C data ~~was~~ were supplied by Dr. C. D. Nelson.

2 Plant 28 did not translocate.

expected from known results of photosynthetically fixed ^{14}C organic compounds.

The opposite leaf was the only area of the soybean plant that contained enough ^{3}H for chromatographic separation of the organic compounds translocated. The sucrose of the opposite leaf contained much less ^{3}H than that of the fed leaf (Table 20). The fed leaf contained equal amounts of ^{3}H in the glucose and fructose but, the opposite leaf contained no ^{3}H in the fructose. Thus, the distribution of ^{3}H in sugars of the opposite and fed leaves was entirely different. The distribution of label in the sugars of the fed leaf was similar whether ^{14}C or ^{3}H was used as tracer. This distribution is typical of sugars formed in photosynthesis. However, the distribution of ^{3}H in the sugars in the opposite leaf is not typical of photosynthesis. The distribution in the opposite leaf is also, not typical of ^{14}C translocation. In ^{14}C translocation almost all the ^{14}C is in sucrose. In ^{3}H translocation very little ^{3}H is in sucrose and most of the ^{3}H is in the amino acids.

It is interesting to note that the distribution of label among the amino acids of the fed leaf is different when ^{3}H and ^{14}C were used. Glutamic acid and glycine were labelled more heavily with ^{3}H than with ^{14}C. These same two amino acids accounted for over 50% of the total ^{3}H translocated to the opposite primary leaf.

Discussion

When ^3H is fed as water the label may exchange with the water in the fed leaf and it may be fixed into organic compounds by photosynthesis. It is expected, that after thirty minutes photosynthesis in ^3H_2O that both the water fraction and the organic compounds in the ethanol-soluble fraction of the fed leaf will be labelled with ^3H. The present work shows that the organic compounds are labelled and in a pattern that is expected from a normal short-term photosynthesis. However, no data ~~has~~ have been gathered concerning the labelling of the water fraction.

Three groups of workers in three different laboratories have studied translocation of ^3H. Biddulph and Cory from the State College of Washington studied translocation of ^3H in kidney bean. Gage and Aronoff from the University of Iowa carried out similar studies with soybean. The present work, carried out at Queen's and Simon Fraser Universities, also used soybean.

Both Biddulph and Cory (2) and Gage and Aronoff (5) collected data on the water fraction and found that it was labelled in the fed leaf. Gage and Aronoff further analyzed the organic compounds and found they contained ^3H, but their techniques did not allow them to separate these compounds to give a distribution pattern of ^3H among the products of photosynthesis.

The translocation of ^{3}H from the fed leaf was shown by all three groups of workers. The water fraction of the leaf, the organic fraction of the leaf or both of these fractions are possible sources of supply for ^{3}H translocated. In other words, ^{3}H may be translocated out of the source leaf either as water or in the form of organic compounds or both.

The assay of sections of plant remote from the fed leaf gives data on the presence or absence of ^{3}H in both water and organic fractions. However such an analysis cannot be used as proof of the form in which ^{3}H is translocated. For example, ^{3}H translocated from the source or fed leaf in the form of water, may exist in the form of water in the stem and leaves or it may be photosynthetically fixed into the organic compounds of the stem and leaves after arrival in these parts. Then, even if water is the form in which ^{3}H is translocated, there may be no ^{3}H in the water fraction of these parts.

Conversely ^{3}H translocated from the source leaf in the form of organic compounds, may exist as such in the stems but may exchange some of its label with the water fraction resulting in both the water and organic fractions of stems being labelled with ^{3}H. One would not expect that such an exchange of label would be so complete as to label the water fraction to the exclusion of the organic fraction.

Biddulph and Cory measured ^3H translocation only in the water fraction of the stem. They found decreasing amounts of ^3H_2O in sections down the stem. The specific activity of the ^3H_2O was not given so the amount of water translocated down the stem could not be determined. However, their results show that ^3H_2O was translocated, and emphasize the importance of determining whether the ^3H found in organic compounds, in parts of the plant other than the fed leaf, are translocated as organic compounds or are translocated as water and subsequently fixed in photosynthesis.

Gage and Aronoff separated the water fraction and organic compounds and measured the content of the translocated ^3H in each fraction. They found ^3H in the organic compounds of the plant parts other than the fed leaf, but no ^3H_2O. Since it is unlikely that a large quantity of ^3H_2O would have been fixed by photosynthesis in the stem, the ^3H must have been translocated in the organic compounds from the fed leaf and not in the form of ^3H_2O. Since the fed leaf contains both ^3H-organic compounds and ^3H_2O the labelled compounds translocated apparently did not mix with the labelled water in the source leaf before this translocation took place.

In the present experiments the ethanol-soluble fraction of parts of the plant other than the fed leaf was found to contain ^3H. The water fraction was not tested for ^3H. However the ^3H was considered to be translocated in the organic fraction on the

basis of the distribution of ^3H in the organic compounds of the fed leaf and in the opposite primary leaf. The ^3H was distributed in the organic compounds of the fed leaf in a pattern typical of photosynthesis; high amounts of sucrose, equal amounts of glucose and fructose, and low amounts of amino acids.

The ^3H-organic compounds formed photosynthetically from ^3H_2O were chromatographed once before (15). Using large amounts of activity and autoradiography, Chlorella was found to contain a large quantity of ^3H-glycollic acid, but little ^3H in other organic compounds. It is interesting that the two carbon compounds, glycollic acid in the experiments with Chlorella and glycine in the experiments with soybean, contained considerable ^3H fixed in photosynthesis.

The ^3H was distributed in the organic compounds of the opposite primary leaf in a different pattern than that obtained in the fed primary leaf. There was a small amount of sucrose, some glucose but no fructose, and high amounts of amino acids. The low amount of sucrose and absence of fructose is not typical of a photosynthetic pattern. Thus, ^3H_2O which might have been translocated to the opposite leaf does not contribute the major part of the ^3H in the organic compounds of the fed leaf. The most probable source for the ^3H amino acids would be the fed leaf. Thus ^3H in the organic compounds of the opposite leaf was translocated as ^3H organic compounds not ^3H_2O. Gage

and Aronoff make the assumption that ^{3}H was translocated as ^{3}H-sucrose from analogy with ^{14}C translocation. Clearly their assumption is not justified. There is some evidence from ^{14}C experiments that amino acids can be translocated in soybean. Under high nitrogen fertilization, serine was translocated from a leaf which had photosynthetically assimilated $^{14}CO_2$ (20).

Gage and Aronoff's experiments were similar to the present experiments in that both of us assayed for ^{3}H in the organic fraction, and both found translocation. Although Gage and Aronoff give the specific activity of the water fed to the leaf, 0.18 mc mmole^{-1} as compared to 1.8 mc mmole^{-1} in the present experiments, they fed water for one hour in the dark previous to photosynthetic assimilation and translocation. They claimed that this technique allowed the water in the leaf to become equilibrated with the water surrounding the leaf. If their claim is correct then the water in the leaf would have a higher specific activity after equilibration had taken place. Since in the present experiments there was no equilibration period the specific activities in both types of experiments are not comparable. There is no way of comparing the amounts translocated when the specific activities of the sources of supply are unknown.

The present experiments show that both the ^{14}C administered as $^{14}CO_2$ and ^{3}H administered as water vapour are photosynthetically fixed in primary leaves of young soybean plants and both isotopes are translocated to other parts of the plant. However, the distribution patterns of the translocated ^{14}C and ^{3}H are different. The ^{14}C is translocated vertically up and down the stem and only slightly to the opposite primary leaf while the ^{3}H is translocated mainly into the opposite leaf.

The translocation of ^{14}C and ^{3}H cannot be compared further. The amount of material represented by amounts of ^{14}C and ^{3}H is determined from specific activities. The specific activities can be determined for the carbon dioxide and water fed to the leaf. However the water present in the leaf will alter the specific activity of the water fed even before it is fixed by photosynthesis. Furthermore, specific activity of the isotope in the organic fraction is no longer an indication of the specific activity of the isotope in the organic compounds as unlabelled organic compounds will dilute the labelled organic compounds. This would not be a factor in comparing translocation of the ^{14}C and ^{3}H if the same organic compound is translocated as the specific activities of the organic compound whether it contained ^{3}H or ^{14}C would be diluted the same amount. This is not the case, however, since ^{14}C is translocated as sucrose and ^{3}H is translocated as amino acids. Since specific activities of the isotope in the organic compounds of the fed leaf and the

translocation stream are unknown, the amount of organic compounds involved in translocation, and represented by movement of ^{14}C and ^{3}H, cannot be determined from the data. The limitation of this data can be understood by considering the amounts of radioactivity involved in translocation of ^{14}C and ^{3}H and the specific activity of the ^{14}C and ^{3}H offered to the fed leaf (Table 21).

The total radioactivity found in the ethanol soluble fraction of the fed leaf is 15 μc for ^{14}C and much less, only 0.006 μc for ^{3}H. However, the total radioactivity offered is 50 μc of ^{14}C and 2500 μc of ^{3}H. The specific activity of ^{14}C is fourteen times the specific activity of ^{3}H when the specific activities are expressed as mc $mmole^{-1}$. This means that the cpm of ^{3}H must be multiplied by fourteen to compare with the cpm of ^{14}C. Of the ^{14}C offered 30% was incorporated into the ethanol-soluble fraction of the fed leaf but only 0.00024% of the ^{3}H offered was incorporated into the same fraction. The factor of fourteen is not enough to bring the 0.00024% for ^{3}H close to the 30% for ^{14}C. However, the specific activity of the ^{3}H fed to the leaf may be diluted by a considerable amount of water already present in the leaf. The water in the leaf would decrease the specific activity of the ^{3}H to an even greater extent and increase the factor of fourteen. From the specific activity the 0.006 μc of ^{3}H in the fed leaf represents 3.3×10^{-6} mmoles or 6.04×10^{-8}g of water. The fresh weight of the leaf is 0.38 g of which over 80% is water. The water fed could

Table 21. Comparison of the amounts of ^{14}C and ^{3}H assimilated and translocated in Stage I soybean plants.

	^{14}C	^{3}H
Total radioactivity offered (μc)	50	2,500
Specific activity (mc mmole^{-1})	26.6	1.8
Total radioactivity in the ethanol-soluble fraction of fed leaf (μc)	15	0.006
Total radioactivity in the ethanol-soluble fraction translocated (μc)	0.1	0.006

be diluted considerably by the water present in the leaf. From this data the dilution factor cannot be determined. Thus, these experiments cannot be used for comparison of the amounts of ^{14}C and ^{3}H incorporated in the fed leaf.

The amount of ^{14}C and ^{3}H fixed in the fed leaf indicates the size of the pool from which organic compounds can be drawn for translocation. The total ^{14}C translocated is 0.67% of the total ^{14}C fixed in the ethanol-soluble fraction of the fed leaf (Table 21). The total ^{3}H translocated is 50% of the total ^{3}H fixed in the fed leaf (Table 21). However, these ratios do not necessarily represent the total amount of organic compounds which are translocated. The specific activity of organic compounds which are translocated must be known before the ^{14}C and ^{3}H translocated can be interpreted to movement of organic compounds. The specific activity of the organic compounds can be decreased by unlabelled compounds already present in the leaf and also unlabelled compounds being translocated from other leaves. Dilution of the organic compound translocated would not be important if the ^{14}C and ^{3}H were translocated in the same compound as the specific activities would be changed by the same amount. However, ^{14}C is translocated as sucrose and ^{3}H appears to be translocated as amino acids. This difference means the specific activity of the organic compounds in the fed leaf and the translocation stream must be known for a comparison of the different amounts of ^{14}C and ^{3}H translocated to the amount of ^{14}C and ^{3}H

in the ethanol-soluble fraction of the fed leaf to be significant. Since this is unknown, no comparison can be made.

The comparison of ^{14}C and ^{3}H translocation required plants which could be expected to have regular and uniform translocation. The Stage 0 and Stage I plants were found to be good material for translocation studies while the Stage II plants were discarded on the basis of only three plants out of five being found to translocate. To have as much grounds as possible for comparison of the plants used as experimental material the weights and leaf areas of the plants were taken as an index of development, the rate of photosynthesis, the total amount of ^{14}C fixed in the ethanol-soluble fraction of the fed leaf and the total ^{14}C translocated were taken as indication of the physiological response of the plants.

Any one physiological response is often considered an indication of the general physiological activity of the plant. However, this may not be the case for all translocation studies. Correlations showed no relationship between the rate of photosynthesis, the total amount of ^{14}C found in the ethanol-soluble fraction of the fed leaf, and the total ^{14}C translocated. Lack of correlation between ^{14}C assimilated and ^{14}C translocated has been reported previously (20). However, it has been reported that the rate of ^{14}C translocation was linearly related to the specific activity of the sucrose in the source leaf in steady state photosynthesis where sucrose was the dominant source of the

transport molecule (6). However, under non-steady state conditions of photosynthesis such as those reported in this work, no correlation exists between the rate of photosynthesis and the rate of translocation, and it is incorrect to use the rate of photosynthesis as an indication of translocation.

Addendum Arising Out of Thesis Defence

Dr. Vidaver questioned whether ^{3}H was assimilated into the organic compounds by photosynthesis or by metabolic steps other than photosynthesis. No dark control of $^{3}H_2O$ was done. In answer to this question it can be pointed out that Gage and Aronoff (5) found no assimilation of ^{3}H into the organic compounds of soybean from $^{3}H_2O$ in the dark. Moses and Calvin (15) found less ^{3}H and a different pattern of label in the organic compounds during a dark feeding of Chlorella. Thus, from previous experiments, ^{3}H is either not assimilated into the organic compounds in the dark, or is assimilated into the organic compounds in different patterns during photosynthesis and in darkness. In the experiments described in this thesis the isotopes were assimilated into the sugars in a similar pattern during both ^{14}C and ^{3}H feedings. The ^{14}C pattern in the sugars is typical of photosynthesis. The pattern of ^{3}H assimilation into amino acids is not typical of photosynthetic assimilation patterns using ^{14}C. The incorporation of ^{3}H from $^{3}H_2O$ vapour may be due to dark fixation. This question remains unanswered.

Summary

1. The primary leaves of young soybean plants photosynthetically fix ^{14}C administered as $^{14}CO_2$ and ^{3}H administered as $^{3}H_2O$ vapour in to the ethanol-soluble fraction of the leaves. However, the distribution patterns of ^{14}C and ^{3}H, translocated to various parts of the plant, are different.
2. Although, ^{14}C is translocated in the form of sucrose, ^{3}H is not translocated as sucrose but appears to be translocated in the form of amino acids.
3. When ^{14}C is fed under conditions of non-steady state photosynthesis, the total ^{14}C translocated in a young soybean plant in thirty minutes does not correlate with the rate of photosynthesis of the fed leaf or with the total ethanol-soluble ^{14}C in the fed leaf.
4. A new technique is described for assaying ^{3}H-labelled compounds taken from paper chromatograms.

Bibliography

1. Ballard, L. A. T. 1941. The represent effect of carbon dioxide on photosynthesis. New Phytologist 40: 276-90.

2. Biddulph, O. and R. Cory. 1957. An analysis of translocation in the phloem of the bean plant using THO, P^{32}, and C^{14}. Plant Physiol. 32: 608-19.

3. Carter, J. L. and E. E. Hartwig. 1963. The management of soybeans. In: The Soybean. A. G. Norman, ed. Academic Press Inc. p. 162-226.

4. Davidson, E. A. 1962. Techniques for paper strip counting in a scintillation spectrometer. Packard Technical Bulletin Number 4.

5. Gage, R. S. and S. Aronoff. 1960. Translocation III. Experiments with C^{14}, Cl^{36}, and H^{3}. Plant Physiol. 35: 53-64.

6. Geiger, D. R. and C. A. Swanson. 1965. Sucrose translocation in the sugar beet. Plant Physiol. 40: 685-90.

7. Hendricks, S. B. and H. A. Borthwick. 1963. Control of plant growth by light. In: Environmental Control of Plant Growth. L. T. Evans, ed. Academic Press Inc. p. 233-63.

8. Hopkins, H. T., A. W. Specht and S. B. Hendricks. 1950. Growth and nutrient accumulation as controlled by oxygen supply to plant roots. Plant Physiol. 25: 193-209.

9. Hull, H. M. 1952. Carbohydrate translocation in tomato and sugar beet with particular reference to temperature effect. Am. J. Botany 39: 661-69.

10. Jenkins, W. A. 1953. Estimating the tritium content of tritiated water. Analytical Chemistry 25: 1477-83.

11. Kramer, P. J. and H. B. Currie. 1950. Water relations of plant cells and tissues. Ann. Rev. Plant Physiol. 1: 265-84.

12. Lister, G. R., G. Krotkov and C. D. Nelson. 1961. A closed circuit apparatus with an infra-red $C0_2$ analyzer and a geiger tube for continuous measurement of $C0_2$ exchange in photosynthesis and respiration. Can. J. Botany 39: 855-64.

13. Mode, E. B. 1961. Elements of Statistics, third edition. Prentice-Hall Inc. p. 164.

14. Mortimer, D. C. 1965. Translocation of the products of photosynthesis in sugar beet petioles. Can. J. Botany 43: 269-80.

15. Moses, V. and M. Calvin. 1959. Photosynthesis studies with tritiated water. Biochem. Biophys. Acta 33: 297-12.

16. Nelson, C. D. and P. R. Gorham. 1957. Uptake and translocation of C^{14}-labelled sugars applied to primary leaves of soybean seedlings. Can. J. Botany 35: 339-47.

17. Nelson, C. D., H. J. Perkins and P. R. Gorham. 1958. Note on a rapid translocation of photosynthetically assimilated C^{14} out of the primary leaf of the young soybean plant. Can. J. Biochem. Physiol. 36: 1277-79.

18. Nelson, C. D. and P. R. Gorham. 1959. Physiological control of the distribution of translocated amino acids and amides in young soybean plants. Can. J. Botany 37: 439-47.

19. Nelson, C. D., H. J. Perkins and P. R. Gorham. 1959. Evidence for different kinds of concurrent translocation of photosynthetically assimilated C^{14} in the soybean. Can. J. Botany 37: 1181-89.

20. Nelson, C. D., H. Clauss, D. C. Mortimer and P. R. Gorham. 1961. Selective translocation of products of photosynthesis in soybean. Plant Physiol. 36: 581-88.

21. Nelson, C. D. 1963. Effect of climate on the distribution and translocation of assimilates. In: Environmental Control of Plant Growth. L. T. Evans, ed. Academic Press Inc. p. 149-74.

22. Norman, A. G. (ed.). 1963. The Soybean. Academic Press Inc.

23. Parker, M. W. and H. A. Borthwick. 1950. Influence of light on plant growth. Ann. Rev. Plant Physiol. 1: 43-58.

24. Parker, M. W., S. B. Hendricks, H. A. Borthwick, and N. J. Scully. 1945. Action spectrum for the photoperiodic control of floral initiation in Biloxi soybean. Science 102: 152-55.

25. Rabineau, G. S. and G. O. Burr. 1945. The use of C^{13} isotope as a tracer for transport studies in plants. Am. J. Botany 32: 349-56.

26. Shiroya, M., G. R. Lister, C. D. Nelson and G. Krotkov. 1961. Translocation of C^{14} in tobacco at different stages of development following assimilation of $C^{14}O_2$ by a single leaf. Can. J. Botany 39: 855-64.

27. Thompson, J. F. and F. C. Steward. 1951. Investigation of nitrogen compounds and nitrogen metabolism in plants II. Variables in 2-directional paper chromatography of nitrogen compounds: a quantitative procedure. Plant Physiol. 26: 421-440.

28. Vernon, L. P. and S. Aronoff. 1952. Metabolism of soybean leaves IV. Translocation from soybean leaves. Arch. Biochem. Biophys. 36: 383-98.

29. Webb, J. A. and P. R. Gorham. 1964. Translocation of photosynthetically assimilated C^{14} in straight necked squash. Plant Physiol. 39: 663-72.

30. Went, F. W. 1953. The effect of temperature on plant growth. Ann. Rev. Plant Physiol. 4: 347-62.

Appendix

Table A 1. Correlation of rate of photosynthesis of fed leaf (x) with the total ^{14}C in ethanol-soluble fraction of the fed leaf (y) in 14-day-old soybean plants (Stage 0). (Data from Table 10).

$$r = \frac{N\Sigma xy - \Sigma x\Sigma y}{\sqrt{[N\Sigma x^2 - (\Sigma x)^2][N\Sigma y^2 - (\Sigma y)^2]}}$$

$N = 9$

$\Sigma x = 1396$

$\Sigma x^2 = 219174$

$\Sigma y = 115.2$

$\Sigma y^2 = 1591.04$

$\Sigma xy = 17568.6$

$r = 0.54$

Conclude: no positive correlation at 5% level of significance.

Table A 2. Correlation of total ^{14}C in ethanol-soluble fraction of the fed leaf (y) with the total ^{14}C translocated (z) in 14-day-old soybean plants (Stage 0). (Data from Table 10).

$$r = \frac{N\Sigma yz - \Sigma y \Sigma z}{\sqrt{\left[N\Sigma y^2 - (\Sigma y)^2\right]\left[N\Sigma z^2 - (\Sigma z)^2\right]}}$$

$N = 9$

$\Sigma y = 115.2$

$\Sigma y^2 = 1591.04$

$\Sigma z = 3413$

$\Sigma z^2 = 1746807$

$\Sigma yz = 44333.4$

$r = 0.0089$

Conclude: no positive correlation at 5% level of significance.

Table A 3. Correlation of rate of photosynthesis of fed leaf (x) with the total ^{14}C translocated (z) in a 14-day-old soybean plant (Stage 0). (Data from Table 10).

$$r = \frac{N\Sigma xz - \Sigma x \Sigma z}{\sqrt{\left[N\Sigma x^2 - (\Sigma x)^2\right]\left[N\Sigma z^2 - (\Sigma z)^2\right]}}$$

$N = 9$

$\Sigma x = 1396$

$\Sigma x^2 = 219174$

$\Sigma z = 3413$

$\Sigma z^2 = 1746807$

$\Sigma xz = 514232$

$r = 0.44$

Conclude: no positive correlation at 5% level of significance

Table A 4. Correlation of rate of photosynthesis of fed leaf (x) with the total ^{14}C in ethanol-soluble function of the fed leaf (y) in 19-day-old plant (Stage I). (Data from Table 11).

$$r = \frac{N\Sigma xy - \Sigma x\Sigma y}{\sqrt{\left[N\Sigma x^2 - (\Sigma x)^2\right]\left[N\Sigma y^2 - (\Sigma y)^2\right]}}$$

$N = 5$

$\Sigma x = 1069$

$\Sigma x^2 = 230579$

$\Sigma = 74.5$

$\Sigma y^2 = 1143.43$

$\Sigma xy = 15889.8$

$r = 0.15$

Conclude: no correlation at 5% level of significance.

Table A 5. Correlation of total ^{14}C in ethanol-soluble fraction of the fed leaf (y) with the total ^{14}C translocated (z) in 19-day-old soybean plants (Stage I). (Data from Table 11).

$$r = \frac{N\Sigma yz - \Sigma y\,\Sigma z}{\sqrt{\left[N\Sigma y^2 - (\Sigma y)^2\right]\left[N\Sigma z^2 - (\Sigma z)^2\right]}}$$

$N = 5$

$\Sigma y = 74.5$

$\Sigma y^2 = 1143.43$

$\Sigma z = 645$

$\Sigma z^2 = 124141$

$\Sigma yz = 9075$

$r = 0.45$

Conclude: no correlation at 5% level of significance

Table A 6. Correlation of rate of photosynthesis of fed leaf (x) with the total ^{14}C translocated (z) in a 19-day-old soybean plant (Stage I). (Data from Table 11).

$$r = \frac{N\Sigma xz - \Sigma x\Sigma z}{\sqrt{\left[N\Sigma x^2 - (\Sigma x)^2\right]\left[N\Sigma z^2 - (\Sigma z)^2\right]}}$$

$N = 5$

$\Sigma x = 1069$

$\Sigma x^2 = 230579$

$\Sigma z = 645$

$\Sigma z^2 = 124141$

$\Sigma xz = 144129$

$r = 0.69$

Conclude: no correlation at 5% level of significance.

Table A 7. Deviation between the two sample means for the rate of photosynthesis between the 14-day-old (x) and 19-day-old plants (y). (Date from Tables 10, 11).

$$t = \frac{(\bar{x} - \bar{y}) - (\mu x - \mu y)}{\sqrt{\left(\frac{NxSx^2 + NySy^2}{Nx + Ny - 2}\right)\left(\frac{1}{Nx} + \frac{1}{Ny}\right)}}$$

$Nx = 9$

$Ny = 5$

$\bar{x} = 155$

$\bar{y} = 214$

$Sx = 18$

$Sy = 18$

$t = 5.44$

Conclude: significant differences at the 5% level

Table A 8. Deviation between the two sample means for the rate of photosynthesis between the 14-day-old plant (x) and the 24-day-old plant (z). (Data from Tables 10, 12).

$$t = \frac{(\bar{x} - \bar{z}) - (\mu x - \mu z)}{\sqrt{\frac{(NxSx^2 + NySy^2)}{(Nx + Ny - 2)} \left(\frac{1}{Nx} + \frac{1}{Ny}\right)}}$$

$Nx = 9$

$Nz = 5$

$\bar{x} = 155$

$\bar{z} = 184$

$Sx = 18$

$Sz = 38$

$t = 2.88$

Conclude: significant difference at the 5% level.

Table A 9. Deviation between the two sample means for the rate of photosynthesis between the 19-day-old (y) and 24-day-old plants (z). (Data from Tables 11, 12)

$$t = \frac{(\bar{y} - \bar{z}) - (\mu x - \mu z)}{\sqrt{\left(\frac{NySy^2 + NzSz^2}{Ny + Nz - 2}\right)\left(\frac{1}{Ny} + \frac{1}{Nz}\right)}}$$

$Ny = 5$

$Nz = 5$

$\bar{y} = 214$

$\bar{z} = 184$

$Sy = 18$

$Sz = 38$

$t = 0.8$

Conclude: no significant difference at 5% level.

Table A 10. Deviation between the two sample means for the total ^{14}C in ethanol-soluble fraction of the fed leaf between the 14-day-old (x) and 19-day-old plants (y). (Data from Tables 10, 11).

$$t = \frac{(\bar{x} - \bar{y}) - (\mu x - \mu y)}{\sqrt{\frac{(NxSx^2 + NySy^2)}{(Nx + Ny - 2)} \left(\frac{1}{Nx} + \frac{1}{Ny}\right)}}$$

$Nx = 9$

$Ny = 5$

$\bar{x} = 12.8$

$\bar{y} = 14.9$

$Sx = 3.6$

$Sy = 2.6$

$t = 1.06$

Conclude: no significant difference at the 5% level.

Table A 11. Deviation between the two sample means for the total ^{14}C in ethanol-soluble fraction of the fed leaf between the 14-day-old (x) and 24-day-old plant (z). (Data from Tables 10, 12).

$$t = \frac{(\bar{x} - \bar{z}) - (\mu x - \mu z)}{\sqrt{\left(\frac{Nx\ Sx^2 + Nz\ Sz^2}{Nx + Nz - 2}\right)\left(\frac{1}{Nx} + \frac{1}{Nz}\right)}}$$

$Nx = 9$

$Nz = 5$

$\bar{x} = 12.8$

$\bar{z} = 18.5$

$Sx = 3.6$

$Sz = 5.5$

$t = 2.2$

Conclude: significant difference at the 5% level.

Table A 12. Deviation between the two sample means for the total ^{14}C in ethanol-soluble fraction of the fed leaf between the 19-day-old (y) and the 24-day-old plant (z). (Data from Tables 11, 12).

$$t = \frac{(\bar{y} - \bar{z}) - (\mu x - \mu z)}{\sqrt{\frac{(NySy^2 + NzSz^2)}{(Ny + Nz - z)} \left(\frac{1}{Ny} + \frac{1}{Nz}\right)}}$$

$Nx = 9$

$Nz = 5$

$\bar{y} = 14.9$

$\bar{z} = 18.5$

$Sy = 2.6$

$Sz = 5.5$

$t = 1.64$

Conclude: no significant difference at the 5% level.

Curriculum Vita

Name:	Robert George Thompson
Place and year of birth:	Almonte, Ontario, 1941.
Education:	Queen's University, Kingston, Faculty of Arts and Sciences 1960-1964, B.Sc. (Biology, Chemistry) 1964. School of Graduate Studies 1964-1965. Simon Fraser University Graduate Studies 1965-1966.
Experience:	Laboratory Demonstrator, Department of Biology, Queen's University, Kingston, 1964-1965. Tutorial Instructor, Department of Biological Sciences, Simon Fraser University, 1965-1966.
Awards:	Government of Ontario Fellowship 1964-1965