# TWO APPROACHES TO THE STUDY OF THE PHYSIOLOGY AND BIOCHEMISTRY OF CHANGES IN TRANSLOCATION DUE TO ENVIRONMENTAL CONDITIONS

by

ROBERT GEORGE THOMPSON

B.Sc. Honours, Queen's University, 1964

M.Sc., Simon Fraser University, 1966

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in the Department

of

Biological Sciences

L.C.



Simon Fraser University

September, 1969

APPROVAL

Name: Robert George Thompson

Degree: Doctor of Philosophy

Title of Thesis: Two approaches to the study of the physiology and biochemistry of changes in translocation due to environmental conditions.

Examining Committee:

D. C. Mortimer
Senior Supervisor
N.R.C. Ottawa

J. H. Borden

G. R. Lister

R. C. Brooke

W. Vidaver

E. B. Tregunna
External Examiner
Associate Professor
Department of Botany U.B.C.
Vancouver

Date Approved: Sept. 29, 1969

# ABSTRACT

Seasonal changes in the rate of translocation in grand fir (Abies grandis Dougl.) were followed using photosynthetically assimilated $^{14}C$-organic compounds and a geiger tube placed against the outside surface of the tree. The geiger tube technique was sensitive enough to identify a bimodal pattern of translocation with peaks in the spring and autumn. Translocation was measured from the tip of branches to regions on the branch closer to the main trunk.

Organic compounds were extracted from the fed region and a non-fed region on the same branch, during the spring, midsummer and autumn. Six hours after the uptake of $^{14}CO_2$ over 80% of the $^{14}C$-organic compounds were recovered from the needles of the fed region. Different amounts of $^{14}C$ were found in the non-fed branch corresponding to the changes in rate of translocation with season. The metabolism of the ethanol-soluble organic compounds to ethanol-insoluble organic compounds increased with increasing rates of translocation, although the relationship was not directly proportional. Sucrose was found to contain most of the $^{14}C$ in the ethanol-soluble compounds of both the fed needles and the non-fed region of the branch except during the spring when there were also large amounts of $^{14}C$-shikimic and $^{14}C$-quinic acids in the fed needles. Thus sucrose appears to be the form in which the bulk of carbon is translocated in grand fir.

Following assimilation of both $^{3}H_2O$ and $^{14}CO_2$ by soybean (Glycine max L.) in the light, similar percentages of non-exchangeable

$^{3}H$ and of $^{14}C$ were distributed in each of the identified compounds with the exceptions of glycine-serine and glutamic acids. Glycine-serine contained proportionally more $^{14}C$ than expected on the basis of percentage of $^{3}H$; and glutamic acid contained proportionally more $^{3}H$ than expected on the basis of $^{14}C$. Both $^{3}H$- and $^{14}C$-organic compounds were translocated at similar rates and in both cases the main compound translocated was sucrose. The metabolism of dark assimilated $^{3}H$ was different from dark assimilated $^{14}C$, but in neither case was it a significant factor in the plant.

Translocation of $^{3}H$- and $^{14}C$-organic compounds under different environmental $O_2$ concentrations was observed after a sequential feeding of $^{3}H_2O$ and $^{14}CO_2$ to the same soybean leaf. At 2% $O_2$ lower translocation rates were observed than at 21% or 99% $O_2$. This was found to be due to the effect of $O_2$ on the distribution of $^{3}H$ and $^{14}C$ among the recent products of photosynthesis.

ACKNOWLEDGEMENT

My basic training and philosophy in the study of plant physiology comes from the late Dr. C. D. Nelson and the late Dr. G. Krotkov. As a graduate student I began my research in plant physiology under Dr. Nelson and at his request continued under Dr. D. C. Mortimer. I am indeed grateful to have known and worked with these three men.

My thanks is expressed to Dr. G. R. Lister for suggestions and discussions which continued my interest in translocation and to Dr. J. H. Borden for carefully reading my manuscript. Dr. R. C. Brooke and many of my fellow graduate students were available for encouragement and sounding out ideas. Drs. G. R. Lister, W. Vidaver and G. H. Geen were most helpful in making the administrative arrangements connected with my graduate program.

To Dr. N. R. Bulley who spent many hours in discussion and reading my rough drafts, I extend a special thanks.

I am most grateful for valuable technical assistance from Dorothy Stotesbury, Janice Alke, Denise Rekdal, Pat Lee and Peter Marshall.

Without the encouragement of my wife Pat this work would not have had any meaning.

TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

Chapter 4

# INTRODUCTION TO THE THESIS

Most of the recent knowledge about translocation of organic compounds in vascular plants has been obtained using $^{14}C$-labelled organic compounds. Translocation rates have been established in many species (1) but because of the difficulties in detecting low levels of $^{14}C$ and because translocation resembles a "speeded-up diffusion process", maximum rates of translocation are not completely agreed upon (7). In fact two types of translocation have been reported, rapid translocation (2000 cm/hr) (9) and slow translocation (100 cm/hr), both functioning in the same plant.

The anatomy of sieve tubes, believed to carry organic compounds (13), is also controversial. Some workers believe that sieve tubes contain cytoplasmic strands stretching from sieve plate to sieve plate through the lumen of the cell and these strands function in translocation in intact cells (4,12). Other workers believe that strands are an artifact of sectioning and are part of an immovable fibril layer attached to the parietal layer of cytoplasm (2,3). Whichever theory of sieve-tube anatomy is proven correct, it will clarify the several theories of phloem translocation which have been proposed to date (6,11,12,15,16).

Although an accurate criterion for maximum rate of translocation and the mechanism by which translocation operates has not yet been established, bulk movement of organic compounds can be measured and compared using $^{14}C$. Beyond the theoretical level of translocation studies, there are many unanswered questions at the level of movement of organic compounds in intact plants. The factors controlling seasonal changes in translocation in trees

(5,10,14) are unknown. These controlling factors are probably related to environmental changes (8) and if they can be regulated perhaps translocation could be stimulated under certain conditions. Another problem is variation in translocation between plants of the same species with the same previous conditioning. Increasing the number of plants to obtain statistically significant results is one method to reduce the problem of individual variation but it would be useful to perform more than one experiment on the same plant. The nature of most translocation experiments using $^{14}C$ necessitates that the plant be used only once since it must be killed and extracted to assay for amounts of $^{14}C$-organic compounds.

With these ideas in mind, translocation in young grand firs was studied with the hope of producing results that could be applied to field application of systemic insecticides to combat the balsam woolly aphid. This problem was approached from the standpoint of determining if there was a seasonal nature to translocation in fir trees, similar to other conifers (5,10,14) and establishing the controlling factors on this seasonal translocation. Because translocation rates between individual trees were variable it was decided to develop a technique using first $^{3}H$ then $^{14}C$-labelled organic compounds under two different environmental conditions in the same plant. Since woody tissue is hard to extract this technique was developed with soybeans. This technique was used to determine the effect of environmental oxygen concentration on translocation in soybean but has not as yet been applied to trees.

## REFERENCES


1. Devlin, R. M. 1966. Translocation of sugars. In: Plant Physiology. Reinhold. New York. pp. 179-210.

2. Escherich, W., and W. Heyser. 1969. Structures of translocating sieve elements. Paper 1298 presented at XI International Botanical Congress. Seattle.

3. Evert, R. F. 1969. Relation of slime substances to strands in sieve elements. Paper 1296 presented at XI International Botanical Congress. Seattle.

4. Fensom, D. S. 1969. Functioning sieve tubes of Heraculum. Paper 1299 presented at XI International Botanical Congress. Seattle.

5. Gordon, J. C., and P. R. Larson. 1968. Seasonal course of photosynthesis, respiration and distribution of $^{14}C$ in young Pinus resinosa trees as related to wood formation. Plant Physiol. 43:1617-1624.

6. Kursanov, A. L. 1963. Metabolism and the transport of organic substances in the phloem. In: Advances in Botanical Research. Vol. I. Ed., R. D. Preston. Academic Press. New York. pp. 209-278.

7. Lawton, J. R. S. 1969. Tracer methods of measuring phloem translocation. Paper 1294 presented at XI International Botanical Congress. Seattle.

8. Lister, G. R., V. Slankis, G. Krotkov and C. D. Nelson. 1967. Physiology of Pinus strobus L. seedlings grown under high or low soil moisture conditions. Annals of Bot. 31:121-132.

9. Nelson, C. D. 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. 36:1277-1279.

10. Shiroya, T., G. R. Lister, V. Slankis, G. Krotkov and C. D. Nelson. 1966. Seasonal changes in respiration, photosynthesis and translocation of the C labelled products of photosynthesis in young Pinus strobus L. plants. Annals of Bot. 30:81-91.

11. Swanson, C. A. 1959. Translocation of organic solutes. In: Plant Physiology: A Treatise, Vol. II. Ed., F. C. Steward. Academic Press. New York. pp. 481-551.

12. Thaine, R. 1964. Long distance transport in plants. World Rev. of Pest Control. 3:175-187.

13. Trip, P., and P. R. Gorham. 1968. Bidirectional translocation of sugars in sieve tubes of squash plants. Plant Physiol. 43:877-882.

14. Ursino, D. J., C. D. Nelson, and G. Krotkov. 1968. Seasonal changes in the distribution of photo-assimilated $^{14}C$ in young pine plants. Plant Physiol. 43:845-852.

15. Wardlaw, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.

16. Zimmermann, M. H. 1960. Transport in phloem. Ann. Rev. Plant Physiol. 11:167-190.

# Chapter 1

## Seasonal Variations in $^{14}C$ Translocation in Grand Fir

# INTRODUCTION

Heavy infestations of balsam woolly aphid (Adelges piceae Ratz.) are destroying economically important stands of true firs (Abies spp.) in the maritime provinces, the Appalachians of northeastern United States, northwestern United States and British Columbia (1,2). The balsam woolly aphid feeds on the bark of balsam fir causing production of abnormal and non-functional phloem which eventually kills the tree (13). It has been suggested that an insecticide might be sprayed on fir trees, absorbed and carried in the translocation stream to the infested region (12). This would be in preference to directly spraying the aphids which are protected by their waxy wool and by crevices in the bark and would protect natural insect predators from contact with the insecticide. A knowledge of translocation and seasonal variations in magnitude and paths of translocation is required before a proper evaluation of systemic insecticides can be made.

A systemic insecticide sprayed onto the surface of the tips of fir branches was found to migrate in the phloem to other parts of the tree (11). If there is a seasonal character to the translocation in the fir, then the correct time to embark on a spray program becomes vital.

There are conflicting reports on seasonal variation of translocation in trees. Seasonal changes in the magnitude of $^{14}$C-photosynthate translocated from the shoot to the root of three-year-old white pine (Pinus strobus L.) (7,8,14) and from new needles to root in red pine (Pinus resinosa Ait.) (4) fits a bimodal curve with peaks in the spring and autumn and little translocation during July and August. Peak translocation of $^{14}$C-photosynthate

has also been reported in the autumn with no peak in the spring from shoots to roots in young white pine (17) and from old needles to roots in red pine (4). Only, a spring peak in translocation of $^{14}C$-photosynthate occurs from new needles to old needles and from new needles to new needles in red pine (4).

It was suggested that the time of slow translocation from the shoots to the roots of white pine can be determined as follows (7):

1. By permitting shoots to carry on photosynthesis in $^{14}CO_2$, extracting and measuring the $^{14}C$ translocated to the roots.

2. By observing the growth-rates of the leader stem and the new needles.

3. By permitting a shoot to carry on photosynthesis in $^{14}CO_2$ for 1 hour and comparing the specific activity of $^{14}CO_2$ respired at the end of feeding as compared to this specific activity 7 hours later.

4. By observing the bio-electric potential difference between the base and top of the seedling.

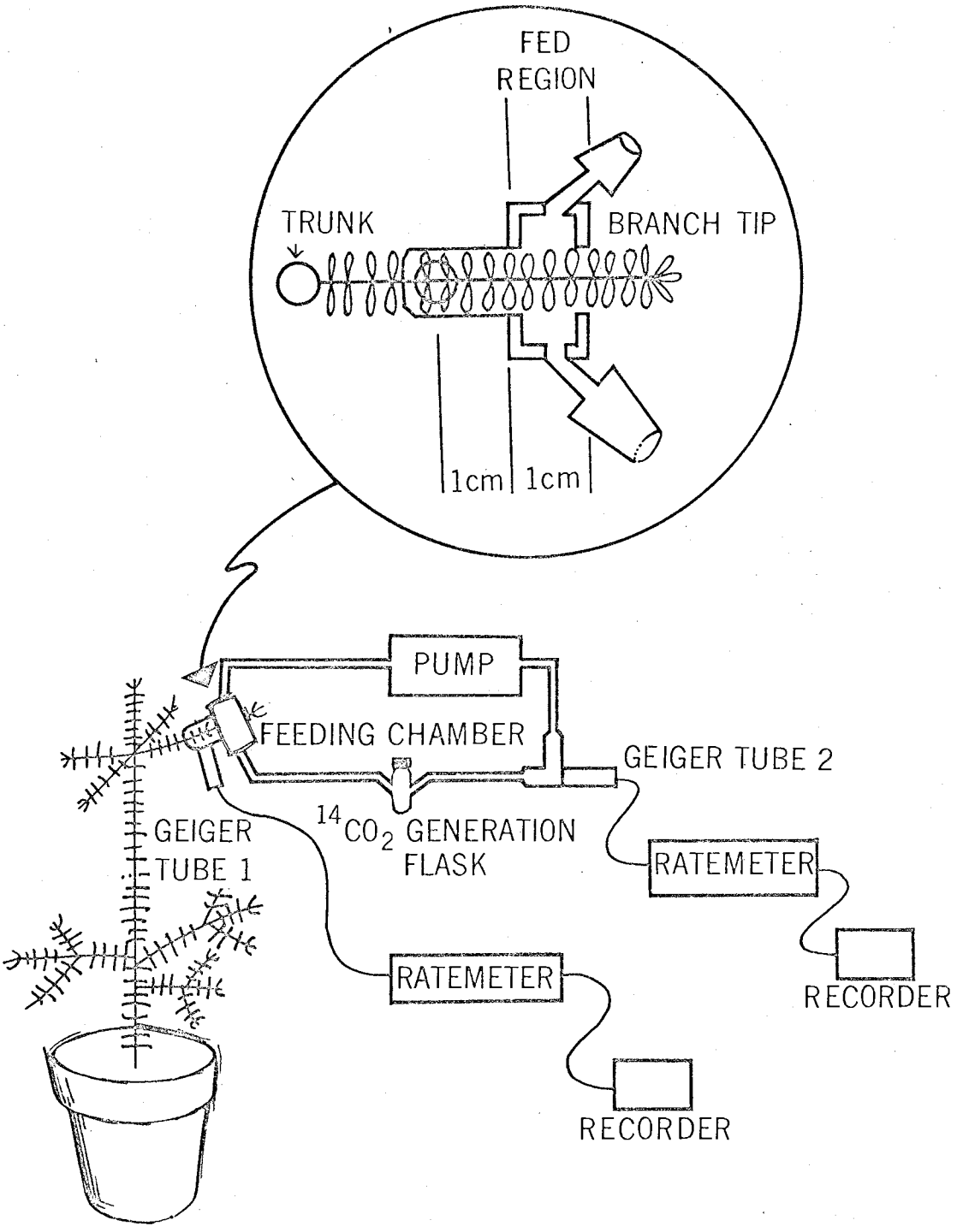
An additional method is the use of a geiger tube to measure the loss of $^{14}C$ from the fed region of a leaf (5) but this method has not been used to directly measure translocation in trees. By feeding $^{14}CO_2$ and using the geiger tube to follow the translocation of recently assimilated products of photosynthesis it should be possible to follow seasonal variations in translocation without destroying the tree. This method could be adapted to field application permitting determination of the physiological activity of the translocation process in a particular stand of fir at a given elevation or location.

## MATERIALS AND METHODS

Grand firs (Abies grandis Dougl.), 20 stock, potted in November 1966 were replanted and left out of doors until taken to the laboratory between May and September 1968. At the beginning of May 1968 branches to be fed $^{14}CO_2$ were selected for uniform size from the top whorl of branches on the trees. On the day before feeding, the trees were placed in a growth cabinet (16 hours of light at 70°, 8 hours of darkness at 56°). Light intensity was 2,000 ft-c as measured by a Weston light meter. At the time of feeding the trees were four-years old and had two whorls of branches, one at the top of the tree and another at the base of the main trunk.

A 1 cm section of the branch tip was sealed (with paraffin wax and vaseline 1:1) in a plexiglass feeding chamber (Fig. 1.1). During feedings from May 16 to June 4 (May-June), the feeding chamber enclosed the branch tip. In feedings during July and September the branch tip was left outside the chamber and a section 1 cm long lower on the branch was enclosed in the chamber. A geiger tube (Anton-Lionel type 6222) was held by a projection from the feeding chamber against the under surface of the branch between the feeding chamber and the main tree trunk. Thus on different trees the geiger tube was placed in constant position 1 cm from the feeding chamber. The geiger tube output was connected to a ratemeter (Nuclear Chicago 8731) and a recorder (Riken Denshi Model S P-J 2). This arrangement measured $^{14}C$ which was transported from the fed region. The feeding chamber was part of a closed gas circuit containing a pump, a $^{14}CO_2$ generating flask and a second geiger tube (Anton-Lionel type 6222). The second geiger tube

Figure 1.1 Apparatus for feeding $^{14}CO_2$ to 1 cm section of grand fir.


FED
REGION
TRUNK
BRANCH TIP
1cm
1cm
PUMP
FEEDING CHAMBER
GEIGER TUBE 2
GEIGER
TUBE 1
$^{14}CO_2$ GENERATION
FLASK
RATEMETER
RECORDER
RATEMETER
RECORDER

with its ratemeter and recorder measured the $^{14}CO_2$ remaining in the closed circuit of the feeding apparatus to indicate uptake of $^{14}C$ by the branch. A 10 µl aliquot of $Na_2{}^{14}CO_2$ (specific activity 76.6 atoms percent, 140 µc) was converted to $^{14}CO_2$ with 2N $H_2SO_4$ and the $^{14}CO_2$ offered to the branch section for 1 hour. At the end of the feeding time the circuit was opened and room air was pumped through the chamber. Care was taken to flush the $^{14}CO_2$ out of the growth cabinet.

From the beginning of feeding until extraction a continuous record lasting for 2, 6 or 24 hours in May-June, 2, 6, 36 or 48 hours in July and 2, 6, 24 or 48 hours in September was kept of the counts per minute (cpm) on the geiger tube outside the fed area. At the end of these times the branch was cut into the fed region, tip of the branch above the fed region, and the branch between the fed region and the main trunk was divided into stem, needles and side branches off the main branch. In feedings during May-June, side branches were included with needles of the main branch. Each piece was weighed, killed in boiling 80% ethanol: 20% water, homogenized (VirTis Homogenizer Model 45) and extracted with hot 80% ethanol: 20% water. The extract was filtered through Whatman No 1 filter paper and the ethanol removed from the extract in a flash evaporator. The extract was diluted with water to a known volume and the amount of $^{14}C$ was assayed in a liquid scintillation spectrometer (Packard Tricarb Series 3000) using a dioxane-based scintillation solution. Counts of $^{14}C$ were corrected for quench (18) with an external standard (Radium 226).

The ethanol-insoluble residue was washed from the filter paper, dried and then converted to $^{14}CO_2$ by wet combustion (9,10,20). The resulting $^{14}CO_2$ was dissolved in a 2:1 mixture of ethylene glycol monomethylether and

methanolamine and its radioactivity determined in a dioxane-based scintillation solution in the scintillation spectrometer.

## RESULTS

In May-June the fresh weights of stem and needles of the fed region was slightly less than later in the season (Table 1.1) since new growth at the tip of the branch was not fully expanded and the chamber enclosed the tip rather than a section further down the branch. Fresh weights of the branch tip and the side branches off the main branch showed the greatest variation (for July feedings 80% and 83% coefficient of variation[1].). Difficulty in placing the chamber so that a uniform amount of the branch was exposed above the tip accounts for variation in fresh weights of the branch tip. Uneven growth of side branches off the main branch accounts for their variability. Fresh weights of the fed region showed the smallest variation (less than 25% coefficient of variation), except for the fed needles in the May-June feedings which showed a larger variation (47% coefficient of variation). This larger variation is due to expanding needles. Since a 1 cm section was always enclosed in the chamber the fed region showed less variation than other sections of the branch.

The amount of $^{14}C$ present in the stem was of the same order of magnitude for all feedings (Table 1.2). Total $^{14}C$ found in the branch at any given time is the amount of $^{14}C$ assimilated into the organic compounds in photosynthesis plus $^{14}CO_2$ lost due to respiration and $^{14}C$-organic compounds lost from the branch by translocation. Others have found (4) that in red pine, respiration during the 72 hours following feeding with $^{14}CO_2$, accounts for the loss of only a small fraction of the total $^{14}C$ applied. It is assumed, in the absence of direct determination

1. coefficient of variation $= \frac{\text{standard deviation}}{\text{mean}} \times 100$

Table 1.1 Fresh weights of pieces of grand fir branches at different periods during the season.

| | May 16-June 4[1]. g | July 8-16[1]. g | Sept. 2-20[2]. g |
|---|---|---|---|
| (branch tip | ——— | 0.15 ± 80% | 0.18 ± 39% |
| (fed needles | 0.13 ± 47% | 0.18 ± 22% | 0.20 ± 10% |
| (fed stem | 0.06 ± 17% | 0.08 ± 25% | 0.09 ± 10% |
| (stem between fed region (and main trunk | 0.11 ± 9% | 0.12 ± 33% | 0.16 ± 13% |
| (needles between fed (region and main trunk | 0.65 ± 31% | 0.52 ± 54% | 0.52 ± 35% |
| (branch off main branch (outside fed region | ——— | 0.24 ± 83% | 0.48 ± 52% |

1. mean of 4 trees ± coefficient of variation.

2. mean of 8 trees ± coefficient of variation.

Table 1.2 Total $^{14}C$ found in the ethanol-soluble and ethanol-insoluble fraction of a branch of grand fir where a 1 cm section of the branch was offered $^{14}CO_2$ for 1 hour and then killed and extracted at 2, 6, 24 and 48 hours after the beginning of feeding.

| Date | time from start of 1 hour feeding to extraction | total $^{14}C$ found in branch dpm |
|---|---|---|
| May 16 | 2 hours 10 min | $47.1 \times 10^6$ |
| May 17 | 5 hours 45 min | 106 |
| June 3 | 2 hours | 64.0 |
| June 4 | 6 hours 10 min | 62.6 |
| | | 69.9 ± 31%[1]. |
| July 8 | 2 hours | 99.7 |
| July 9 | 6 hours | 76.6 |
| July 15 | 2 hours | 132 |
| July 16 | 6 hours | 57.6 |
| | | 91.6 ± 30% |
| Sept 2 | 2 hours | 76.8 |
| Sept 3 | 6 hours | 103 |
| Sept 5 | 2 hours | 114 |
| Sept 6 | 6 hours | 95.6 |
| | | 97.4 ± 14% |
| Sept 9 | 24 hours | 59.1 |
| Sept 10 | 24 hours | 39.6 |
| Sept 16 | 48 hours | 74.3 |
| Sept 18 | 48 hours | 53.1 |
| | | 56.5 ± 22% |

1. mean ± the coefficient of variation.

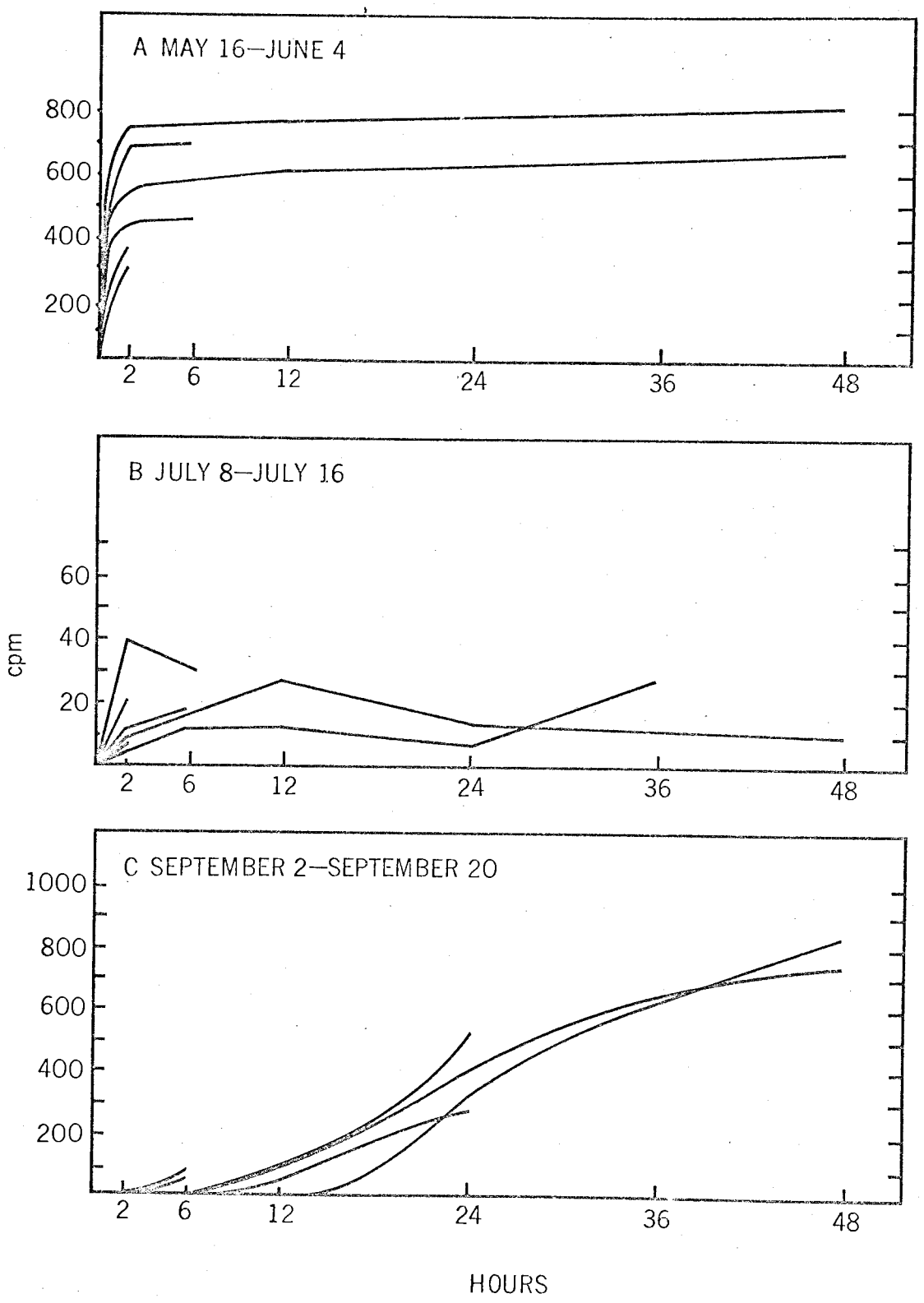
that the grand fir responds not too differently. Translocation of $^{14}C$ from the branch to other parts of the tree also contributes to loss of $^{14}C$. The extent of this loss will be evaluated in a later section.

For May-June, July and September the 2 and 6 hour feedings were treated as a group to determine if there were any statistically significant differences between the amount of $^{14}C$ left in the branch during the different seasons. There is no significant difference (t-test, P=0.05) between the average amounts of $^{14}C$ found in the branches 2 and 6 hours after the feeding began for any of the seasonal periods studied. The average amount of $^{14}C$ in branches after 24 and 48 hours in September is significantly less (t-test, P=0.01) than after 2 and 6 hours in September. This loss of $^{14}C$ could be accounted for by a greater time for respiration and translocation out of the branch.

In the May-June feedings there was a more rapid increase in $^{14}C$ in the branch outside the fed region during the 1 hour feeding than later in the season (Fig. 1.2). This increase levelled off before the end of the second hour after feeding began. This was followed by a period of slow increase in $^{14}C$ which continued steadily for at least 48 hours. In July feedings there was only a small increase (40 cpm) in the amount of $^{14}C$ measured outside the fed region during the feeding, and the amount of $^{14}C$ did not increase with time.

In September feedings, an increase in the amount of $^{14}C$ outside the fed region began between 2 and 14 hours after the start of feeding. A steady increase continued until there was about the same amount of $^{14}C$ after 48 hours in September (Fig. 1.2C) as after 6 hours in May-June (Fig. 1.2A).

Figure 1.2 Seasonal changes in rate of translocation of $^{14}C$ to a region 1 cm closer to the main trunk than the fed region. The $^{14}C$ was measured with a geiger tube placed against the lower surface of the branch.


A MAY 16—JUNE 4
800
600
400
200
2
6
12
24
36
48
B JULY 8—JULY 16
cpm
60
40
20
2
6
12
24
36
48
C SEPTEMBER 2—SEPTEMBER 20
1000
800
600
400
200
2
6
12
24
36
48
HOURS

The efficiency of the $^{14}C$ measurement can be expressed by the value obtained on an instrument (cpm) divided by the amount of $^{14}C$ present in the sample measured (dpm). The geiger tube was less than 1% efficient at measuring total $^{14}C$ (Table 1.3). In July feedings the geiger tube measured the small amount of $^{14}C$ with a greater efficiency but with a variation of over 100% as compared to variation of less than 30% in May-June or September. The geiger tube reflected the amounts of ethanol-soluble $^{14}C$-organic compounds with less variation than the amounts of ethanol-insoluble $^{14}C$-organic compounds. There is no difference in efficiency values when ethanol-soluble $^{14}C$ replaces total $^{14}C$ in the efficiency calculations. When the ethanol-soluble $^{14}C$ is compared with the cpm from the geiger tube there is an increase in variation to between 60% and 130%.

Appendix 1 shows efficiency values obtained with the geiger tube for counting $^{14}C$-organic compounds on chromatography paper and dried on a glass cover slip. The geiger tube was more efficient and showed less variation in assaying $^{14}C$ under these conditions.

At the end of 2 hours, needles in the fed region contained about 90% of the total $^{14}C$ in the branch and showed no signs of losing $^{14}C$ even after 6 hours (Fig. 1.3A). September feedings extracted after 48 hours showed a slight decrease in $^{14}C$ to about 85% (Fig. 1.3A).

In May-June feedings the percentage of $^{14}C$ found in the stem of the fed region decreased from 2 to 6 hours (Fig. 1.3B). There was a smaller percentage of the $^{14}C$ in the stem of the fed region in July than in May-June or September. In September feedings there was a large variation in the two values for the 2 hour experiment, and the average percentage of $^{14}C$ found

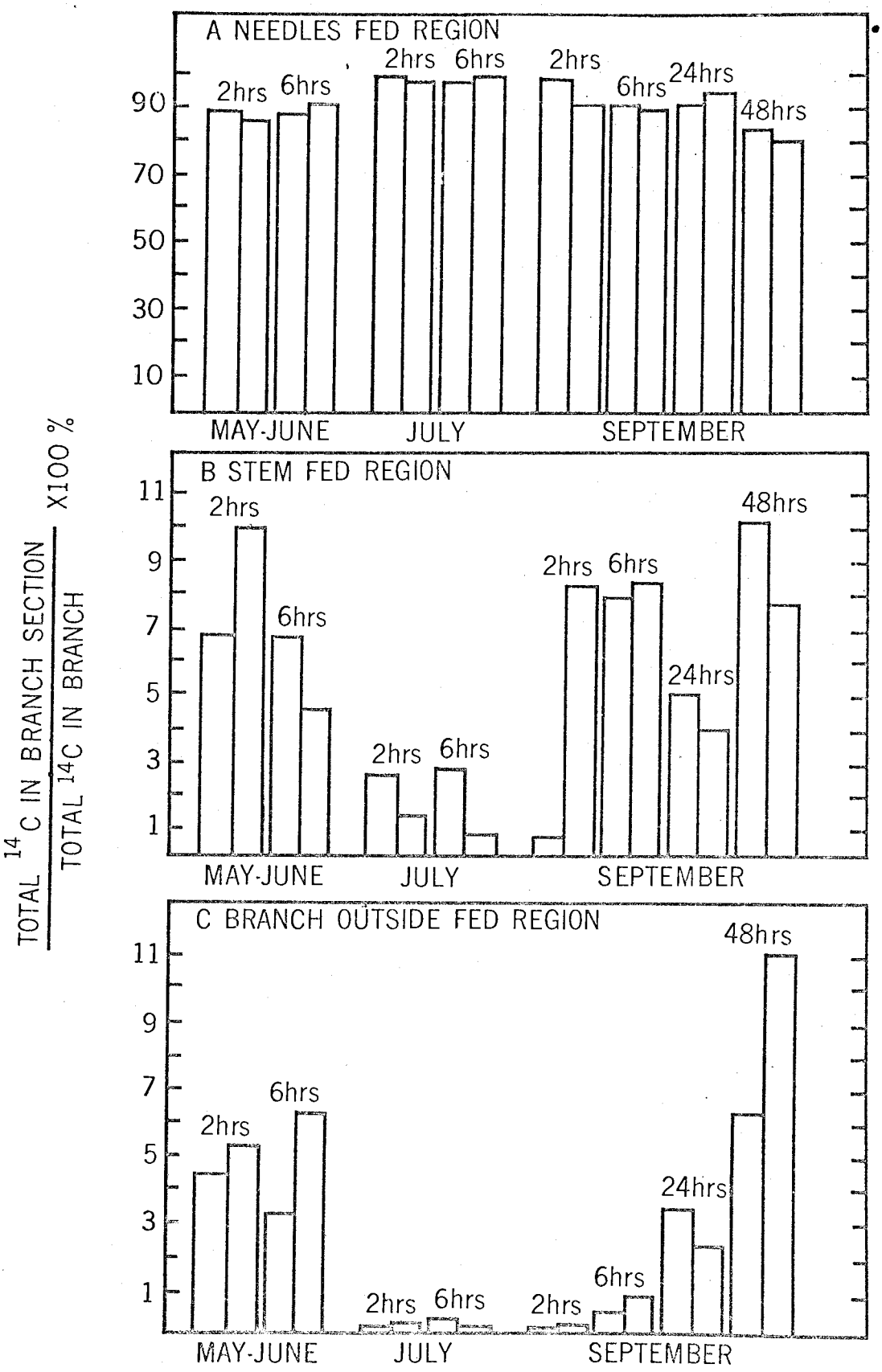
Table 1.3 The efficiency of the geiger tube in measuring ethanol-soluble $^{14}C$, ethanol-insoluble $^{14}C$ and total $^{14}C$ (ethanol-soluble plus ethanol-insoluble) in the grand fir branch outside the fed region at different periods during the season.

| | $\frac{\text{cpm geiger tube}}{\text{total } ^{14}C} \times 100$ | $\frac{\text{cpm geiger tube}}{\text{ethanol-soluble } ^{14}C} \times 100$ | $\frac{\text{cpm geiger tube}}{\text{ethanol-insoluble } ^{14}C} \times 100$ |
|---|---|---|---|
| May-June[1]. | 0.031 ± 19% | 0.031 ± 19% | 4.20 ± 62% |
| July[1]. | 0.125 ± 110% | 0.128 ± 110% | 12.9 ± 120% |
| Sept[2]. | 0.046 ± 33% | 0.041 ± 29% | 3.64 ± 130% |

1. mean of 4 trees ± coefficient of variation.

2. mean of 8 trees ± coefficient of variation.

Figure 1.3 Percentage of total $^{14}C$ (ethanol-soluble plus ethanol-insoluble) distributed among different sections of the branch. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.


A NEEDLES FED REGION
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
90
70
50
30
10
MAY-JUNE
JULY
SEPTEMBER
B STEM FED REGION
11
9
7
5
3
1
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
C BRANCH OUTSIDE FED REGION
11
9
7
5
3
1
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
TOTAL $^{14}$C IN BRANCH SECTION / TOTAL $^{14}$C IN BRANCH X100 %

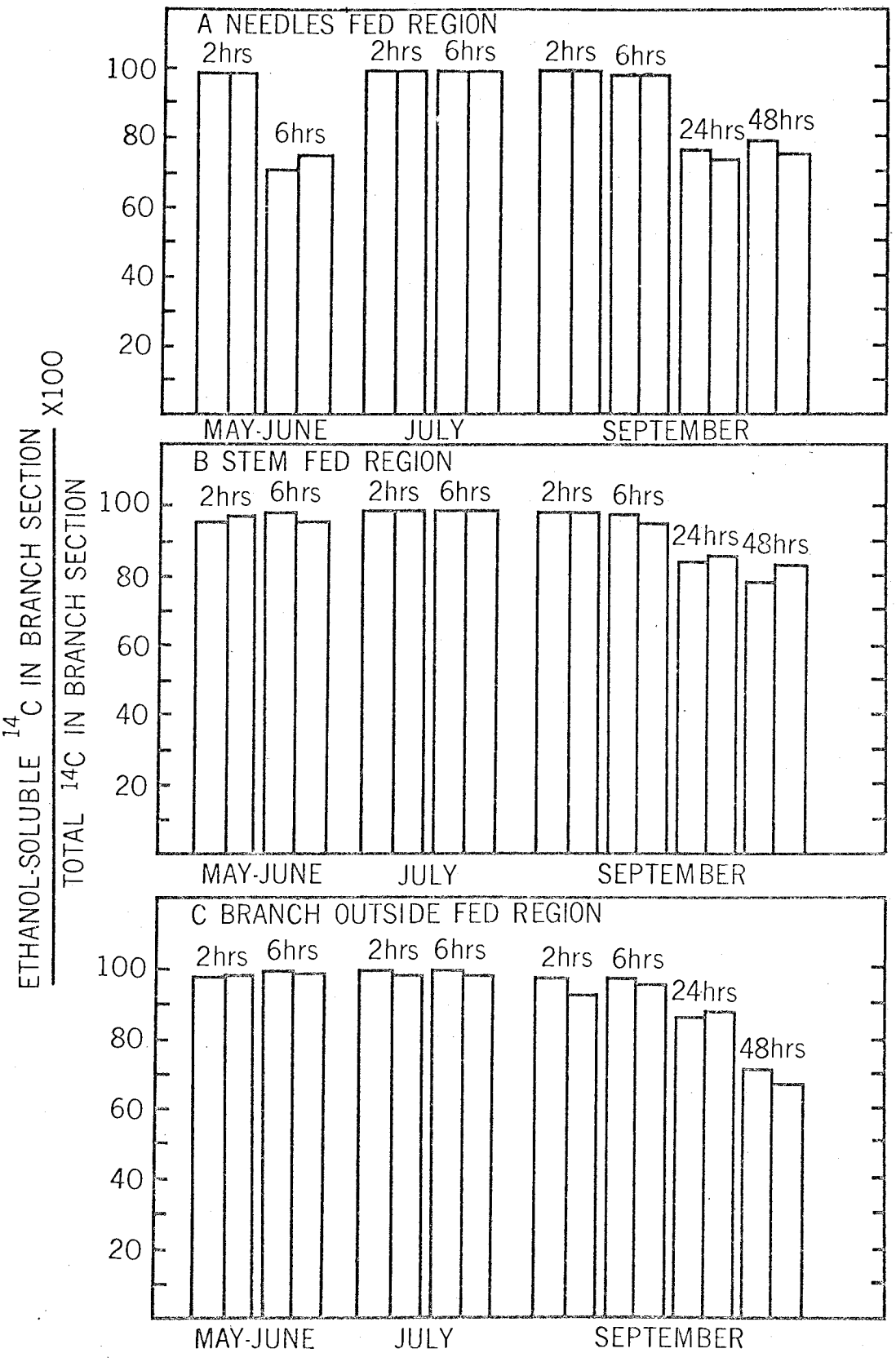
in the stem of the fed region increased from 2 to 6 hours. However, if only the highest value after 2 hours is used, the percentage of $^{14}C$ found in the stem remained constant between 2 and 6 hours. From 6 hours to 24 hours the percentage of $^{14}C$ found in the stem decreased but increased from 24 hours to 48 hours.

In May-June and September feedings the percentage of $^{14}C$ found in the branch outside the fed region increased from 2 to 6 hours (Fig. 1.3C). There was no increase during July. May-June feedings showed the larger rate of increase. The slower rate of increase in the September feedings continued to 48 hours after the start of feeding. This seasonal change in rate of increase of amount of $^{14}C$ found in the branch outside the fed region corresponds to seasonal changes found with the geiger tube.

Feedings during May-June showed a decrease in percentage of ethanol-soluble $^{14}C$-organic compounds of total $^{14}C$-organic compounds in the needles from 2 to 6 hours (Fig. 1.4A). This represents a corresponding increase in ethanol-insoluble $^{14}C$-organic compounds. There was no decrease with time in ethanol-soluble $^{14}C$-organic compounds in samples examined after 2 and 6 hours in July and September. Feedings during September showed a decrease in ethanol-soluble $^{14}C$-organic compounds at 24 hours and 48 hours (Fig. 1.4A).

There was no decrease in the ethanol-soluble $^{14}C$-organic compounds of the stem of the fed region from 2 to 6 hours in any of the feedings (Fig. 1.4B). In September there was a decrease from 6 hours to 24 and 48 hours. The branch outside the fed region showed a decrease in percentage of ethanol-soluble $^{14}C$-organic compounds from 6 to 24 hours and a further

Figure 1.4 Ethanol-soluble $^{14}C$-organic compounds expressed as a percentage of total $^{14}C$ (ethanol-soluble plus ethanol-insoluble) of different sections of the branch. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.


A NEEDLES FED REGION
2hrs
2hrs 6hrs
2hrs 6hrs
6hrs
24hrs 48hrs
100
80
60
40
20
MAY-JUNE
JULY
SEPTEMBER
B STEM FED REGION
2hrs 6hrs
2hrs 6hrs
2hrs 6hrs
24hrs 48hrs
100
80
60
40
20
MAY-JUNE
JULY
SEPTEMBER
C BRANCH OUTSIDE FED REGION
2hrs 6hrs
2hrs 6hrs
2hrs 6hrs
24hrs
48hrs
100
80
60
40
20
MAY-JUNE
JULY
SEPTEMBER
ETHANOL-SOLUBLE $^{14}C$ IN BRANCH SECTION / TOTAL $^{14}C$ IN BRANCH SECTION X100

decrease from 24 to 48 hours in September (Fig. 1.4C) but there was no significant decrease between 2 and 6 hours at any period in the season.

## DISCUSSION

To follow seasonal variations trees must be similar in size and age and they should be subjected to seasonal environmental changes. These criteria were met by selecting branches similar in size and position on the tree at the beginning of the season. Until the day before feeding and except for potting the trees were kept out of doors under normal seasonal environmental conditions.

The larger amounts of $^{14}C$ measured by the geiger tube in May-June and September feedings compared to July feedings is open to several interpretations. An increased amount of $^{14}C$ measured by the geiger tube could indicate an increased amount of $^{14}C$ accumulation, not an increased amount of $^{14}C$ moving past it during translocation. Also an increased amount of $^{14}C$ measured by the geiger tube could indicate an increased photosynthetic assimilation of $^{14}C$ and more $^{14}C$-organic compounds to translocate not just an increased rate of translocation.

Seasonal fluctuation in photosynthetic activity does not seem to explain the differences in translocation measured with the geiger tube. Since similar amounts of $^{14}C$ were found in the fed branch after 2 and 6 hours from time of feeding (Table 1.2), photosynthetic assimilation of $^{14}C$ in May-June was not much greater compared to July and September. Thus more $^{14}C$ available for translocation in May-June does not explain the greater amount of $^{14}C$ in the region over the geiger tube after 2 and 6 hours in May-June as compared to the same length of time in July or September (Fig. 1.2). It might be argued that the $^{14}C$ is assimilated

more quickly in May-June but the excess $^{14}C$ assimilated is completely translocated out of the fed branch by a corresponding increase in translocation. However previous studies of the whole tree indicate that over 90% of the ethanol-soluble $^{14}C$ is in the fed region or the region next to the fed region even after 48 hours (Appendix 2). In the branch studies, ethanol-soluble $^{14}C$ closely parallels ethanol-insoluble $^{14}C$ in distribution although there is always more ethanol-soluble $^{14}C$ than ethanol-insoluble $^{14}C$ (Fig. 1.4). Thus only a small amount (less than 10%) of the $^{14}C$ should occur outside the fed branch in the present studies. This is not enough $^{14}C$ for a greater rate of photosynthesis to explain the increased translocation rate in May-June. In white pine at the end of 8 hours of peak translocation following 1 hour when the entire shoot was offered $^{14}CO_2$, no more than 26% of the total $^{14}C$ was exported to the root (7). Even if 26% of the $^{14}C$ was translocated from the grand fir branch in the May-June feedings this is not enough increase in assimilation to account for the increased translocation in May-June (Fig. 1.2).

The geiger tube measures the total amount of $^{14}C$ in the branch but does not distinguish between $^{14}C$ that is moving and the $^{14}C$ that is accumulated in the branch. Accumulation of $^{14}C$ could occur so deep in the branch that the remainder of the branch shielded it from the geiger tube. Since translocation of $^{14}C$-organic compounds occurs in the phloem (reviews by Kursanov (6), Thaine (16), Wardlaw (19), Zimmermann (21)) in the bark of conifers (3,15) it is unlikely translocating $^{14}C$ is shielded completely from the geiger tube. The amount of translocating $^{14}C$ in front of the geiger tube at any one time depends on the velocity and magnitude

of translocation. Once $^{14}C$ is accumulated in the region to which the geiger tube is sensitive it continues to be recorded by the geiger tube. Thus while a large proportion of the $^{14}C$ recorded by the geiger tube may be accumulated $^{14}C$, it is more likely that the record at any one time is a composite of the two vectors. For example in Fig. 1.2, the May-June records probably indicate moving $^{14}C$ during the first 2 hours but after this time the reading represents accumulated $^{14}C$. In the September feedings, however, the recorded value continued to increase for at least 48 hours, which is a clear indication that export into this region of the stem was still going on.

The efficiency of the geiger tube to measure $^{14}C$ in the branch is low for a variety of reasons: the thickness of the branch shields the $^{14}C$ from the geiger tube, the geiger tube measures $^{14}C$ on only the bottom side of the branch, the branch covered only a small area across the middle of the geiger tube and the branch is a cylinder with only a small amount of the surface directly against the geiger tube. In spite of this, the $^{14}C$ detected was sufficient to separate and identify three seasonal translocation patterns in the fir.

Following translocation patterns with a geiger tube involves only a minimum amount of equipment and this technique could easily be adapted to field studies. It would yield as much practical information on the physiology of plants in field conditions as more sophisticated equipment. One practical application would be a field study at different seasons of the year of the translocation of systemic insecticides labelled with radioactive isotopes.

## REFERENCES


1. Balch, R. E., and R. C. Mitchell. 1967. Balsam wooly aphid Adelges (Dreyfusia, Chermes) piceae (Ratz.). In: Forest Insects and Diseases of North America. Eds., A. G. Davidson and R. M. Prentice. Dept. of Forestry and Rural Development Canada. Pub. No. 1180 pp. 70-74.

2. Balch, R. E. 1952. Studies of the balsam woolly aphid, Adelges piceae (Ratz.), and its effects on balsam fir, Abies balsamea (L.) Mill. Can. Agr. Pub. 867.

3. Esau, K. 1953. Plant Anatomy. Wiley, New York. p. 294.

4. Gordon, J. C., and P. R. Larson. 1968. Seasonal course of photosynthesis, respiration and distribution of $^{14}C$ in young Pinus resinosa trees as related to wood formation. Plant Physiol. 43:1617-1624.

5. Hofstra, G. 1967. An approach to the study of physiological control of translocation in higher plants. Ph.D. Thesis. Simon Fraser Univ., Brit. Columbia.

6. Kursanov, A. L. 1963. Metabolism and the transport of the organic substances in the phloem. In: Advances in Botanical Research Vol. I. Ed., R. D. Preston. Academic Press. London. pp. 207-278.

7. Lister, G. R., V. Slankis, G. Krotkov and C. D. Nelson. 1967. Physiology of Pinus strobus L. seedlings grown under high or low soil moisture conditions. Annals of Bot. 31:121-132.

8. Nelson, C. D. 1964. The production and translocation of photosynthate-$^{14}C$ in conifers. In: The Formation of Wood in Forest Trees. Ed., M. H. Zimmermann. Academic Press. New York. pp. 243-257.

9. Rapkin, E. 1962. Measurement of $^{14}CO_2$ by scintillation techniques. Packard Tech. Bull. No. 7. Packard Instrument Co., Illinois.

10. Rapkin, E. 1963. The determination of radioactivity in aqueous solutions. Packard Tech. Bull. No. 6. Packard Instrument Co., Illinois.

11. Rediske, J. H., and N. E. Johnson. 1965. The absorption and translocation of the systemic insecticide schradan in sitka spruce and grand fir. Weyerhaeuser Forestry Paper No. 5. Centralia, Wash.

12. Rudinsky, J. A. 1959. Systemics in the control of forest insects. J. For. 57:284-286.

13. Smith, F. H. 1967. Effects of balsam woolly aphid (Adelges piceae) infestations on cambial activity in Abies grandis. Amer. J. Bot. 54:1215-1223.

14. Shiroya, T., G. R. Lister, V. Slankis, G. Krotkov and C. D. Nelson. 1966. Seasonal changes in respiration, photosynthesis and translocation of the C labelled products of photosynthesis in young Pinus strobus L. plants. Annals of Bot. 30:81-91.

15. Srivastava, L. M. 1964. Anatomy, chemistry and physiology of bark. Int. Rev. For. Res. 1:203-277.

16. Thaine, R. 1964. Long distance transport in plants. World Rev. Pest Control 3:175-187.

17. Ursino, D. J., C. D. Nelson, and G. Krotkov. 1968. Seasonal changes in the distribution of photo-assimilated $^{14}C$ in young pine plants. Plant Physiol. 43:845-852.

18. Wang, C. H., and D. L. Willis. 1965. Measurement of radioactivity by the liquid (internal-sample) scintillation method. Chapt. 6. In: Radiotracer Methodology in Biological Science. Prentice-Hall, N. J.

19. Wardlaw, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.

20. van Slyke, D. O., J. Plazin, and J. R. Wersinger. 1951. Reagents for the van Slyke-Folch wet combustion. J. Biol. Chem. 191:299-304.

21. Zimmermann, M. H. 1960. Transport in phloem. Ann. Rev. Plant Physiol. 11:167-190.

# Chapter 2

## Seasonal Variations in Distribution of $^{14}C$ among the Organic Compounds of the Fed Needles and the Stem Outside the Fed Region in Grand Fir

# INTRODUCTION

Little is known about the control mechanism governing translocation in plants. Biochemical regulation of translocation might be indicated by the availability of products of photosynthesis at the source and the use of these products at the sink, coupled with seasonal changes in translocation patterns. Any information related to the control mechanism of translocation might then be used in increasing the effectiveness of a systemic insecticide.

Various organic phosphorous and fluorine compounds with insecticide properties are absorbed and translocated in forest trees (10). Translocation of these systemic insecticides may be closely related and controlled in a similar manner to translocation of food material in the tree. Schradan labelled with $^{32}P$ was found mainly confined to the phloem tissue in the lower stem after application to the terminal bud of grand fir (9). Translocation of the herbicide 2,4-dichlorophenoxyacetic acid, also an organic compound, was initiated by the presence of sucrose, the compound translocated in kidney beans (3,5). Since the distinction between herbicide and insecticide is mainly functional, transport of systemic insecticides could well resemble transport of systemic herbicides.

When the systemic insecticides, systox and metasystox were sprayed on the foliage of balsam fir in the spring they were found effective against balsam woolly aphid (Wachtendorf (17) as reported by Rudinsky (10)). Spring effectiveness of the insecticide could correspond to increased feeding of the adult woolly aphid on the translocation stream (10) or to increased translocation of the insecticide in the spring (Chap. 1) or a

combination of both these factors. If seasonal variation in translocation is responsible for the effectiveness of insecticides it becomes very important for the proper use of systemic insecticides to understand the mechanisms which control translocation. The present study on grand fir was made to determine if the products of photosynthesis in the needles of the fed region (source) or the compounds accumulated in the stem (sink) were related to seasonal changes in amounts of $^{14}C$ translocated.

# MATERIALS AND METHODS

The ethanol-soluble extracts of the grand fir branches described in Chapter 1 were separated into individual compounds. Extracts were analyzed from the needles of the fed region and the stem just outside the fed region, taken during the two peak times for translocation in May-June and September, and during the low translocation period in June.

These extracts were separated into sugars, amino acids and organic acids using resin column chromatography - Rexyn 101 (H) and 201 (OH) (11). Aliquots of the sugars, amino acids and organic acids were assayed for $^{14}C$ in the liquid scintillation spectrometer (Packard Series 3000) using a dioxane-based scintillation solution. The sugars, amino acids and organic acids were further separated into individual compounds by two dimensional paper chromatography (11). Individual spots were identified on the paper chromatogram using autoradiography and aniline-phthalate, ninhydrin and silver nitrate sprays for the sugars, amino acids and organic acids respectively. The amount of $^{14}C$ in each spot was determined with a methane-flow, window, Geiger-Mueller, detector tube (Nuclear Chicago Model 470).

# RESULTS

Seasonal changes occurred in the distribution of $^{14}C$ in the amino acids and organic acids in the ethanol-soluble extract of the fed needles (Fig. 2.1). In May-June feedings, $^{14}C$ in the ethanol-soluble compounds was split almost evenly between the sugars and the organic acids with less than 2% in the amino acids. In July and September feedings over 90% of the $^{14}C$ in the ethanol-soluble compounds was found in the sugars with small amounts in the organic acids and amino acids. There was less $^{14}C$ in the ethanol-soluble compounds after 24 and 48 hours as compared to after 2 and 6 hours in the September feedings. In July feedings there was less $^{14}C$ in the ethanol-soluble compounds after 6 hours as compared to after 2 hours. In May-June feedings the amount of $^{14}C$ in the ethanol-soluble compounds did not decrease between 2 and 6 hours.

In the stem outside the fed region (at all times during the season) over 90% of the $^{14}C$ in the ethanol-soluble fraction was found in sugar (Fig. 2.2). Less than 2% of the $^{14}C$ appears in the amino acids and less than 10% in the organic acids. At no time during the season did the percentage distribution of $^{14}C$ among the ethanol-soluble compounds of the stem show a significant change with time. Seasonal differences in the amount of $^{14}C$ in ethanol-soluble compounds of the stem fit seasonal changes in translocation (Fig. 1.3C).

Analysis of sugars in the ethanol-soluble compounds of the fed needles (Fig. 2.3) indicated that sucrose contains most of the $^{14}C$ in the sugars during the season. Only 50% of the $^{14}C$ in the sugars was in sucrose

Figure 2.1 Distribution of $^{14}C$ among sugars, amino acids and organic acids of the ethanol-soluble compounds of the fed needles. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.

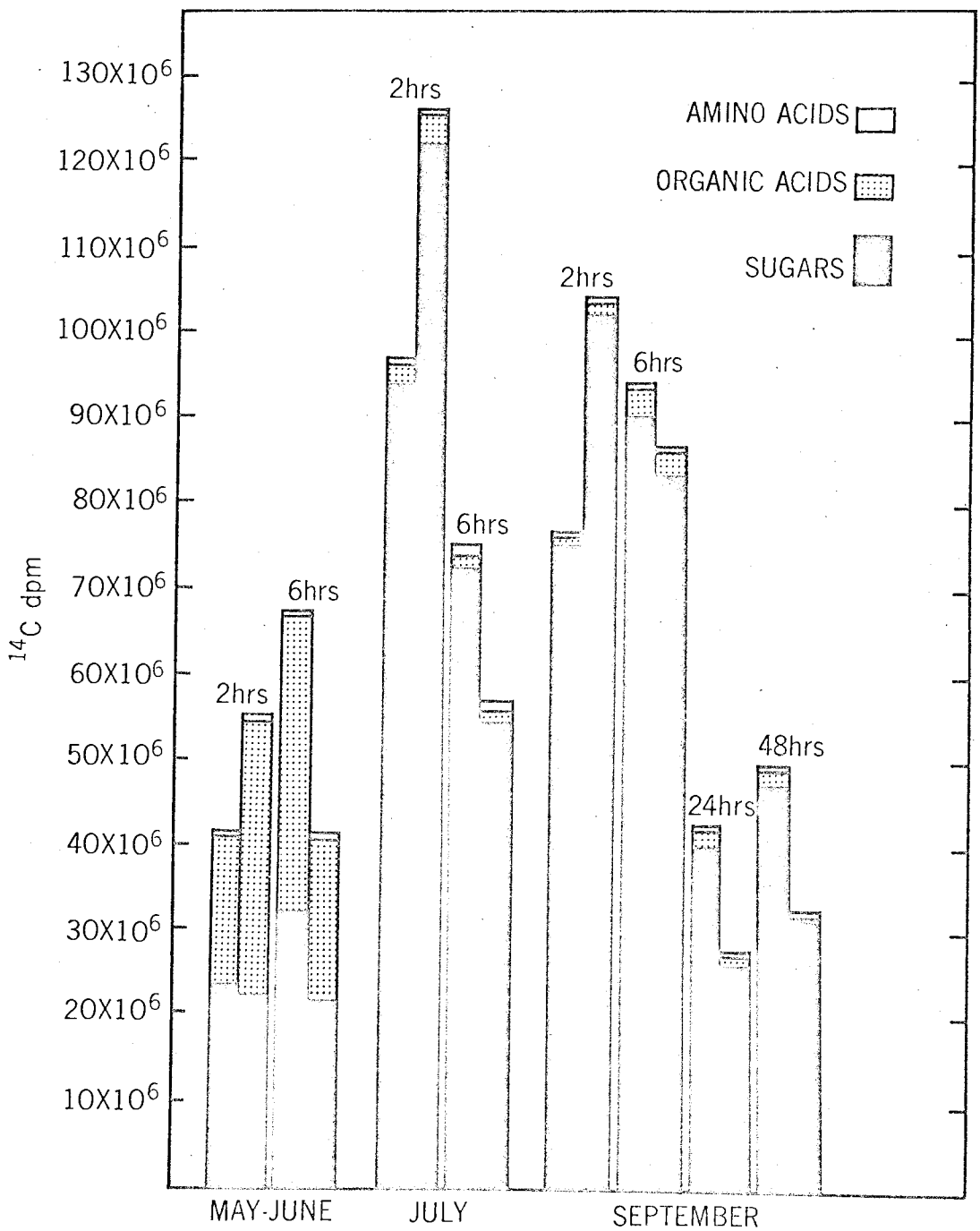

130X10⁶
120X10⁶
110X10⁶
100X10⁶
90X10⁶
80X10⁶
70X10⁶
60X10⁶
50X10⁶
40X10⁶
30X10⁶
20X10⁶
10X10⁶
¹⁴C dpm
AMINO ACIDS
ORGANIC ACIDS
SUGARS
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER

Figure 2.2 Distribution of $^{14}C$ among sugars, amino acids and organic acids of the ethanol-soluble compounds of the stem outside the fed region. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.

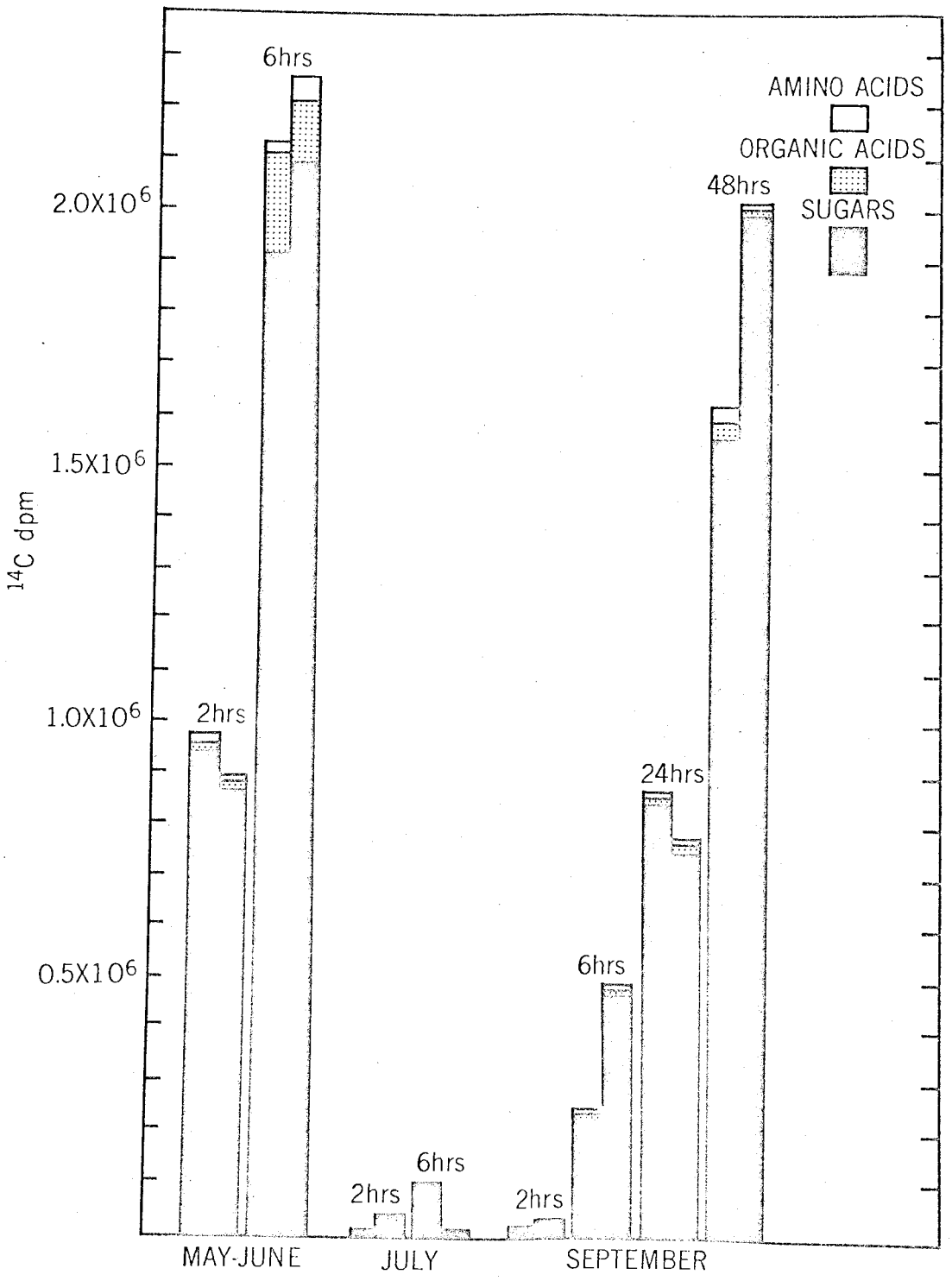
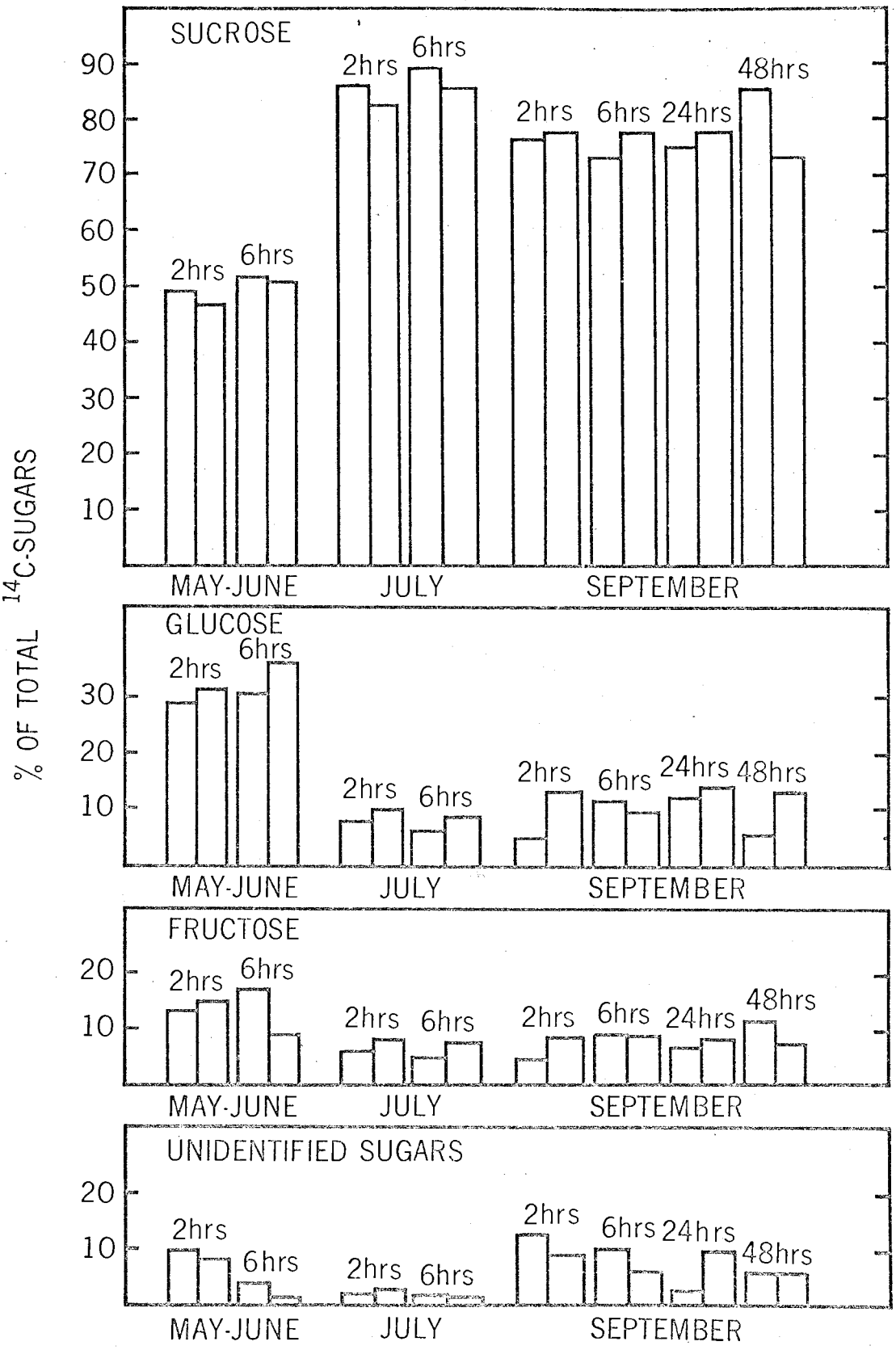

AMINO ACIDS
ORGANIC ACIDS
SUGARS
$^{14}$C dpm
2.0X10$^6$
1.5X10$^6$
1.0X10$^6$
0.5X10$^6$
6hrs
2hrs
48hrs
24hrs
6hrs
6hrs
2hrs
2hrs
MAY-JUNE
JULY
SEPTEMBER

Figure 2.3 Percentage of $^{14}C$ distributed among the sugars of the fed needles. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.

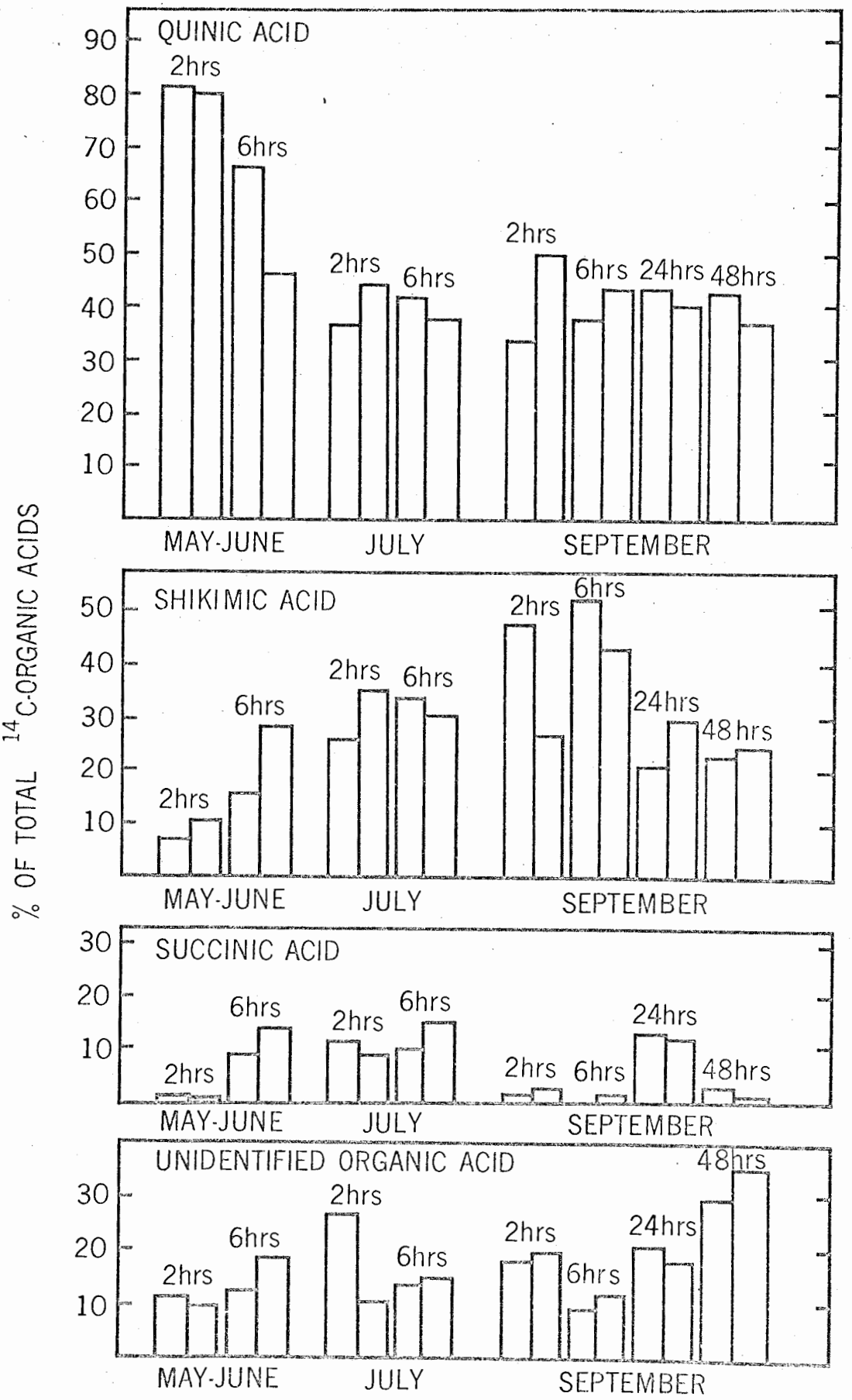

% OF TOTAL $^{14}$C-SUGARS
SUCROSE
90
80
70
60
50
40
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
GLUCOSE
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
FRUCTOSE
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
UNIDENTIFIED SUGARS
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER

in May-June feedings compared to between 80 to 90% in July feedings and 70 to 80% in September feedings. Most of the remaining $^{14}C$ in the sugar fraction was split between glucose and fructose. A higher amount of $^{14}C$ was found in glucose than in fructose in May-June feedings. Small amounts of $^{14}C$ (always less than 10% of the sugar fraction) were found in unidentified sugars and at the origin of the chromatogram. During May-June, July and September feedings there was little change in the distribution of $^{14}C$ in the sugars with time.

Analysis of the organic acids indicated that in May-June feedings quinic acid contained about 80% of the $^{14}C$ in the organic acids after 2 hours and from 45 to 65% after 6 hours (Fig. 2.4). About 40% of the $^{14}C$ in the organic acids was found in quinic acid in July and September feedings. Shikimic acid and succinic acid also contained a large percentage of the $^{14}C$ in the organic acids. In red pine a large amount of the ethanol-soluble $^{14}C$ was also found in quinic acid and shikimic acids, 6 days following a feeding with $^{14}CO_2$ (8). After 6 days no large amount of succinic acid was found in red pine. In grand fir, between 10% and 20% of the $^{14}C$ in the organic acids was split between numerous unidentified organic acids and the origin of the chromatogram except in September feedings after 48 hours when 30% to 35% was found in these compounds. At all seasons the number of organic acids containing small amounts of $^{14}C$ increased with time.

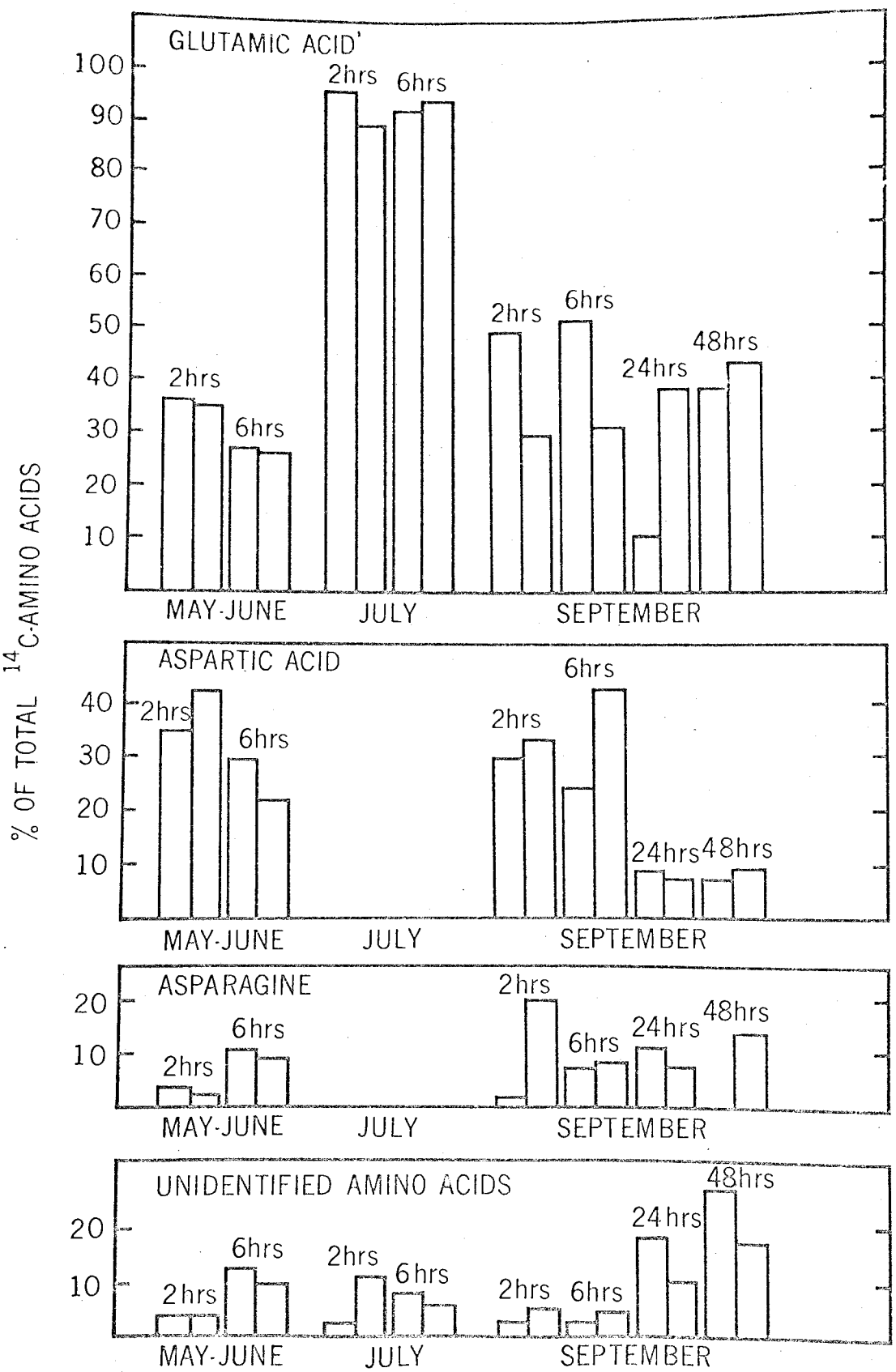
Glutamic acid contained more $^{14}C$ than any other amino acid in the ethanol-soluble extract from the needles of the fed area, having approximately 30% of the $^{14}C$ in the May-June feedings, over 90% in the

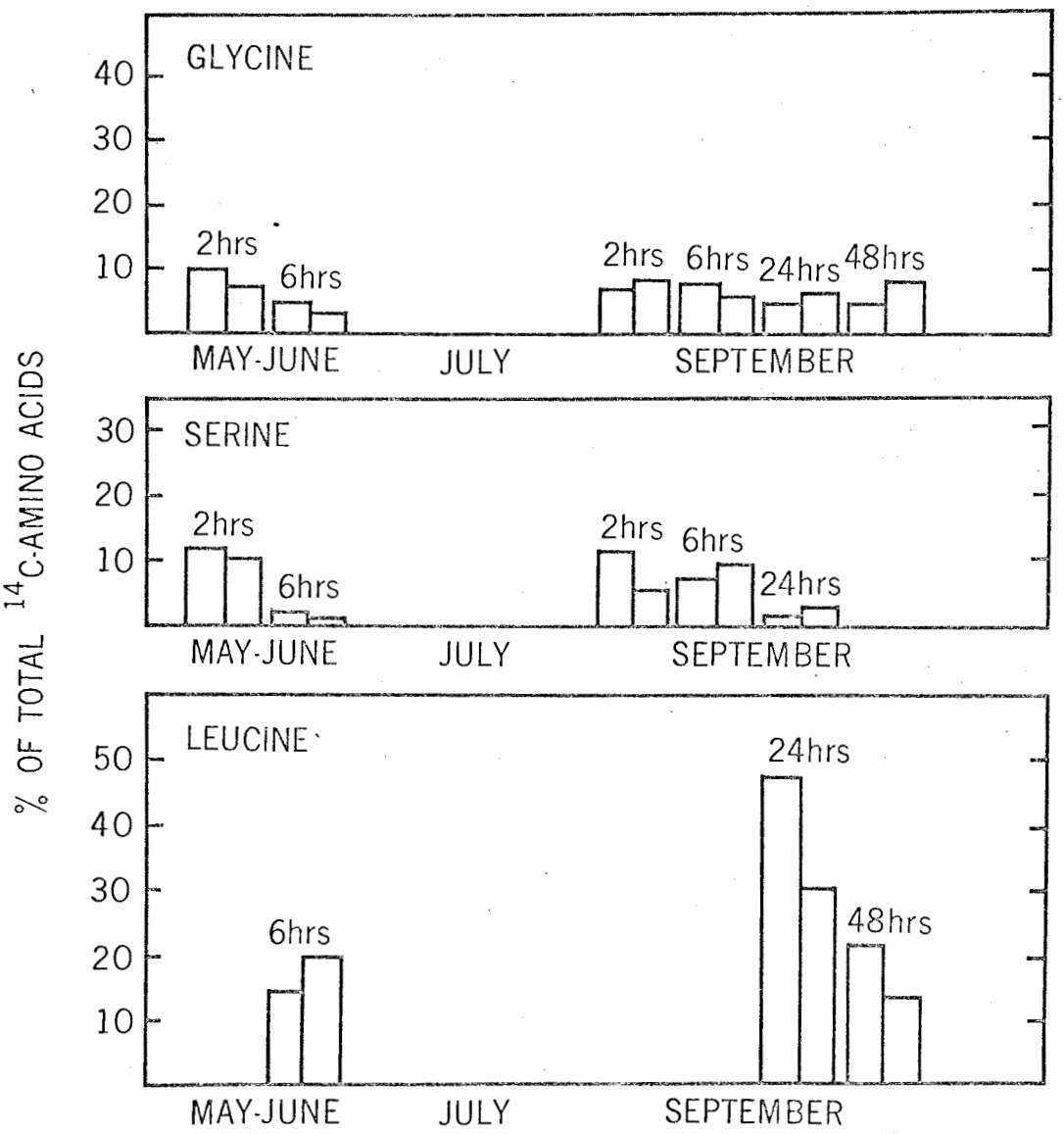
Figure 2.4 Percentage of $^{14}C$ distributed among the organic acids of the fed needles. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.


% OF TOTAL $^{14}$C-ORGANIC ACIDS
QUINIC ACID
90
80
70
60
50
40
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
SHIKIMIC ACID
50
40
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
SUCCINIC ACID
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
UNIDENTIFIED ORGANIC ACID
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER

July feedings and between 10% to 50% in the September feedings (Fig. 2.5). Between 20% and 40% of the $^{14}C$ in the amino acids was found in aspartic acid in the May-June feedings. In the September feedings the percentage of $^{14}C$ in aspartic acid decreased from 20% to 40% at 2 and 6 hours to less than 10% at 24 and 48 hours. Change in percentage of $^{14}C$ also indicates a change in amount of $^{14}C$ in an amino acid since there is no significant difference in the amount of $^{14}C$ found in the amino acid fraction at different periods in the season or at different times from the beginning of feeding (Fig. 2.1). In July feedings all the $^{14}C$ was found in glutamic acid except for a small amount at the origin of the chromatogram. In May-June feedings the amount of $^{14}C$ in aspartic acid decreased from over 30% after 2 hours to between 20% to 30% after 6 hours. In September feedings there was a decrease with time in the amount of $^{14}C$ in aspartic acid after 24 hours rather than after 6 hours as in the May-June feedings. In May-June and September feedings the amount of $^{14}C$ in asparagine was about 10% or less of the total $^{14}C$ in the amino acids. In these feedings the amount of $^{14}C$ in glycine was also about 10%. After 2 hours in May-June and September feedings about 10% of the $^{14}C$ in the amino acids was found in serine. After 6 hours in May-June and after 24 hours in September the percentage of $^{14}C$ in serine had fallen to about 1%. No $^{14}C$ was found in serine after 48 hours in September. In May-June feedings no $^{14}C$ was found in leucine after 2 hours but $^{14}C$ in leucine increased to 15% to 20% of the amino acid $^{14}C$ after 6 hours. Similarly in September feedings no $^{14}C$ was found in leucine after 2 hours and 6 hours but leucine contained between 30% and 50% of the $^{14}C$ after 24 hours followed by a reduction to 10% and

Figure 2.5 Percentage of $^{14}C$ distributed among the amino acids of the fed needles. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.

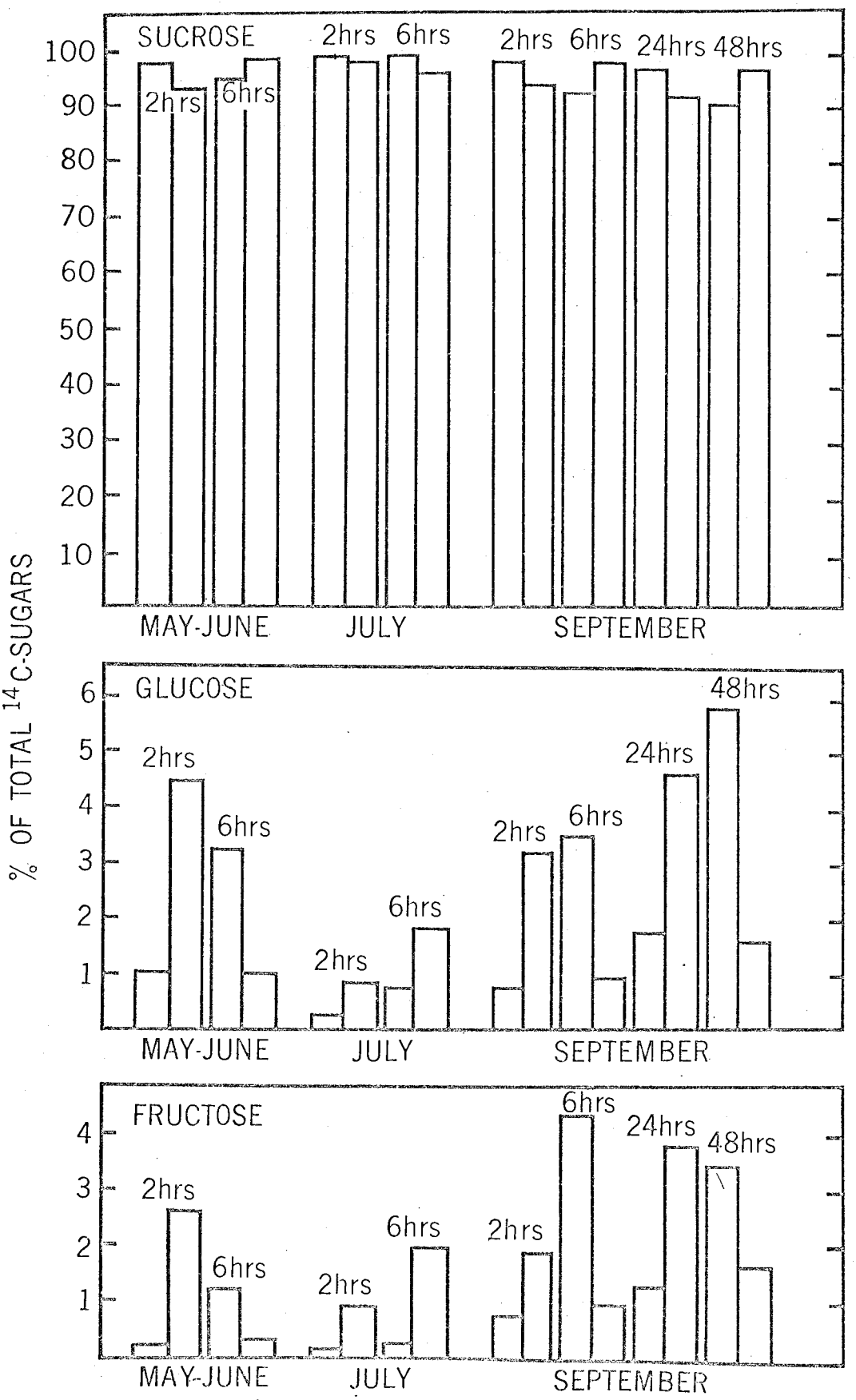

% OF TOTAL $^{14}$C-AMINO ACIDS
GLUTAMIC ACID
100
90
80
70
60
50
40
30
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
ASPARTIC ACID
40
30
20
10
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
ASPARAGINE
20
10
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
UNIDENTIFIED AMINO ACIDS
20
10
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER


% OF TOTAL $^{14}$C-AMINO ACIDS
GLYCINE
40
30
20
10
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER
SERINE
30
20
10
2hrs
6hrs
2hrs
6hrs
24hrs
MAY-JUNE
JULY
SEPTEMBER
LEUCINE
50
40
30
20
10
6hrs
24hrs
48hrs
MAY-JUNE
JULY
SEPTEMBER

20% after 48 hours. After 2 and 6 hours in May-June, July and September feedings less than 15% of the $^{14}C$ in the amino acid fraction was found at the origin of the chromatogram or in numerous unidentified amino acids. After 24 and 48 hours between 15% and 30% of the $^{14}C$ in amino acids was found at the origin of the chromatogram and in unidentified amino acids.

Sugars of the ethanol-soluble fraction of the stem contained enough $^{14}C$ to separate into individual compounds by paper chromatography. Over 90% of the $^{14}C$ was found in sucrose and the rest of the $^{14}C$ was split between glucose and fructose (Fig. 2.6). Distribution of $^{14}C$ between glucose and fructose for individual trees showed a similar pattern i.e. when the percentage of $^{14}C$ in glucose was high, the percentage of $^{14}C$ in fructose was also high. In July and September feedings the distribution of $^{14}C$ between the sugars of the fed area (Fig. 2.3) was the same as the distribution in the stem (Fig. 2.6). This was not true in May-June. About 50% of the $^{14}C$ in the sugar fraction of the needles of the fed area was in sucrose and in the stem over 90% of this $^{14}C$ was in sucrose.

Figure 2.6 Percentage of $^{14}C$ distributed among the sugars of the stem outside the fed region. Each tree (represented by a bar histogram) was offered $^{14}CO_2$ for 1 hour, then left in air for 2, 6, 24 or 48 hours from the beginning of feeding.


SUCROSE
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
100
90
80
70
60
50
40
30
20
10
MAY-JUNE
JULY
SEPTEMBER
% OF TOTAL 14C-SUGARS
GLUCOSE
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
6
5
4
3
2
1
MAY-JUNE
JULY
SEPTEMBER
FRUCTOSE
2hrs
6hrs
2hrs
6hrs
2hrs
6hrs
24hrs
48hrs
4
3
2
1
MAY-JUNE
JULY
SEPTEMBER

# DISCUSSION

Differences between $^{14}C$ compounds found in the fed needles and those found in the stem correlate the differences between assimilated compounds which may or may not be available for translocation as compared with the compounds that are translocated. The compounds in the stem represent accumulation in the stem of translocated compounds and any possible compounds derived from translocated compounds. In all plants in which $^{14}CO_2$ has been used to label the photosynthetically assimilated organic compounds, $^{14}C$ is found in the sugars, amino acids and organic acids, components of the ethanol-soluble and ethanol-insoluble compounds. In herbaceous plants the $^{14}C$ in the ethanol-insoluble compounds is found in cellulose, hemicellulose and starch (6). Cellulose, hemicellulose and starch are also found in trees as well as aromatic compounds such as lignin, coumarins, flavenoids, aromatic alkaloids and tannins (2,13,14). In trees lignin contains $^{14}C$ after feeding with selected precursors such as $^{14}C$-shikimic acid (7), $^{14}C$-mannitol (16) and $^{14}CO_2$ (1). Quinic acid and shikimic acid contain over 60% of the $^{14}C$ in the organic acids in balsam fir (Fig. 2.4). These organic acids are on the pathway towards lignin (7). Unless the polysaccharides and lignin are broken down to their components they are unavailable for translocation. Only a few organic compounds are known to translocate (4,15,18,19) thus many ethanol-soluble compounds are also unavailable for translocation.

Since over 90% of the ethanol-soluble $^{14}C$ in the stem was always found in sugars (Fig. 2.2) translocated compounds in grand fir are most

likely sugars. The translocated sugar is most likely sucrose since 90% of the $^{14}C$ in sugar in the stem was in sucrose (Fig. 2.6). Equal percentages of glucose and fructose in the stem (Fig. 2.6) suggest the hexoses arise from hydrolysis of translocated sucrose. Many other plants also translocate sucrose (4,15,18,19).

Distribution of $^{14}C$ in the organic compounds found in fed needles in balsam fir (Fig. 2.1) is similar to the distribution found in the old shoots of white pine (11). However, no difference in the distribution of $^{14}C$ between organic acids and sugars was noted for white pine during the peak period of translocation but young shoots of white pine were not included in the ethanol-soluble extract. The decrease of $^{14}C$ in sugar and increase in organic acids (Fig. 2.1) in balsam fir occurred in young needles. Distribution of $^{14}C$ among sucrose, glucose and fructose is similar for extracts from old shoots of white pine (11) and in July and September feedings for the current year's needles of balsam fir (Fig. 2.3).

During May-June the period of rapid translocation in balsam fir, the amount of $^{14}C$ in sugars was lower and the amount of $^{14}C$ in organic acids was higher than during the periods of slower translocation. Less sugar and more organic acids in the fed needles could result from: (a) increased translocation of sucrose (b) more sugars converted to structural polysaccharides (14) or (c) a different pattern of $^{14}C$ assimilation in the spring.

About 6% of the $^{14}C$ in the branch was found outside the fed region in the May-June feedings (Fig. 1.3, Chapt. 1). Only a small amount of $^{14}C$ was found in the ethanol-soluble organic compounds outside the fed branch

(Appendix 2). In May-June feedings translocation from the fed region was probably no more than 10% of the $^{14}C$ assimilated in the fed region. Thus translocation of sucrose can account for only a small amount of the decrease in sucrose found in May-June feedings.

Conversion of sugar to structural polysaccharides might explain the lower amount of $^{14}C$ in the ethanol-soluble sugars in May-June feedings. In the spring the amount of $^{14}C$ in the ethanol-insoluble compounds increased from 2% of the total $^{14}C$ in the fed area to about 30% after 6 hours (Fig. 1.4, Chap. 1). Since the distribution of $^{14}C$ between sugars and organic acids was similar at both 2 hours and 6 hours the conversion of ethanol-soluble to ethanol-insoluble compounds should have occurred before 2 hours if it were to account for the greater amount of $^{14}C$ found in organic acids and the smaller amount of $^{14}C$ found in sugars after 2 hours in the May-June feedings. After 6 hours the conversion of 30% of the ethanol-soluble to ethanol-insoluble compounds might partially account for the lower amount of $^{14}C$ found in the sugars but would not account for the increased amount of $^{14}C$ found in organic acids. After 48 hours in September, 30% of the ethanol-soluble compounds were converted to ethanol-insoluble compounds. However at this time there was no increase in percentage of $^{14}C$-organic acids. Thus conversion of sugars to polysaccharides does not completely explain the lower amount of $^{14}C$ in sugars and increased amount of $^{14}C$ in the organic acids found in the May-June feedings.

Since neither increased translocation of sucrose nor increased conversion of "free" sugar groups to polysaccharides can completely account for the difference in proportions of sugars to organic acids found in May-June

there appears to be a distinctly different pattern of assimilation of carbon into organic compounds in the spring. This pattern is characterized by a greater production of organic acids composed mainly of quinic and shikimic acids. Rangnekar and Forward (8) showed that six days following a feeding of $^{14}CO_2$ to the shoot, quinic and shikimic acids contain 23% of the total $^{14}C$ found in the organic compounds of the bud of a red pine seedling. They believe that in the bud of the red pine, quinic and shikimic acids are formed from sucrose and are designated for the synthesis of lignin in the growing shoot apex. In the spring the shoot of balsam fir is growing more rapidly than later in the season and the assimilation of more $^{14}C$ into quinic and shikimic acids would provide more of the precursors of lignin (7) which could be used for cell wall formation. Whether quinic and shikimic acids are formed from sucrose or from another precursor could be determined with feedings of shorter duration.

A relationship between the increased translocation in the spring and the increased proportions of organic acids of the ethanol-soluble organic compounds can be only a speculation on the basis of these experiments. Rapid growth during the spring could account for a greater demand for food material and thus greater translocation of organic compounds. More organic acids for production of lignin for cell walls could also be a response to more rapid growth. However increased translocation and the increased proportion of organic acids may be more closely related. Perhaps the level of organic acids, promotes cell wall elongation and makes the cells more permeable to organic compounds. Thus more sugars "leak" out of the cells to increase translocation. Addition of organic acids to a systemic

insecticide might promote the translocation of the insecticide much as the addition of sucrose promoted the translocation of 2,4-dichlorophenoxyacetic acid in kidney beans (3,5).

The sugars assimilated in the fed needles and accumulated in the stem from the translocation stream contained over 90% of the $^{14}C$ in the ethanol-soluble fraction except in fed region in the May-June feedings where 40% of the $^{14}C$ was found in organic acids. In May-June the magnitude of translocation was highest, needles were youngest and organic acid production was highest. In July and September translocation was lower and needles were more mature. Since extraction occurred at relatively long times from the beginning of feeding, short term changes in the relative distribution of $^{14}C$ among the organic compounds might have been lost. These short term changes in the relative distribution of $^{14}C$ among the organic compounds would indicate changes in rate of metabolic conversion between organic compounds. Metabolism could control translocation by making more compounds available for translocation, converting compounds available for translocation to ones that are not translocated or by making more energy available for translocation (4). The different amount of $^{14}C$ distributed between the sugars and the organic acids in May-June compared to July and September feedings may be significant in relation to greater magnitude of translocation in May-June. However it is impossible to determine if these changes result in greater translocation or are the result of greater translocation. Short term feedings could make this distinction by demonstrating whether or not $^{14}C$-sugars are converted to $^{14}C$-organic acids during the first two hours after assimilation in May-June feedings.

## REFERENCES


1. Balatinecz, J. J., D. F. Forward and R. G. S. Bidwell. 1966. Distribution of photoassimilated $^{14}CO_2$ in young jack pine seedlings. Can. J. Bot. 44:362-365.

2. Jensen, W., K. E. Fremer, P. Sierila and V. Wartiovaara. 1963. The chemistry of bark. In: The Chemistry of Wood. Ed., B. L. Browning. Interscience Pub. N. Y. pp. 587-666.

3. Jaworski, E. G., S. C. Fang and V. H. Freed. 1955. Studies in plant metabolism V. The metabolism of radioactive 2,4-D in etiolated bean plants. Plant Physiol. 30:272-275.

4. Kursanov, A. L. 1963. Metabolism and the transport of organic substances in the phloem. In: Advances in Bot. Res. Vol. I. Ed., R. D. Preston. Academic Press. N. Y. pp. 209-278.

5. Mitchell, J. W., and J. W. Brown. 1946. Movement of 2,4-dichlorophenoxyacetic acid stimulus and its relation to the translocation of organic food materials in plants. Bot. Gaz. 107:393-407.

6. Mortimer, D. C., and C. B. Wylam. 1962. The incorporation of $C^{14}$ into cellulose and other polysaccharides of sugar beet leaf during short term photosynthesis in $C^{14}O_2$. Can. J. Bot. 40:1-11.

7. Neish, A. C. 1964. Cinnamic acid derivatives as intermediates in the biosynthesis of lignin and related compounds. In: The Formation of Wood in Forest Trees. Ed., M. H. Zimmermann. Academic Press. N. Y. pp. 219-239.

8. Rangnekar, P. V., and D. F. Forward. 1969. Foliar nutrition and growth in red pine: the fate of photoassimilated carbon in a seedling tree. Can. J. Bot. 47:897-906.

9. Rediske, J. H., and N. E. Johnson. 1965. The absorption and translocation of the systemic insecticide schradan in sitka spruce and grand fir. Weyerhaeuser For. Paper No. 5. Centralia, Wash.

10. Rudinsky, J. A. 1959. Systemics in the control of forest insects. J. Forestry. 57:284-286.

11. Shiroya, T., V. Slankis, G. Krotkov and C. D. Nelson. 1962. The nature of photosynthate in Pinus strobus seedlings. Can. J. Bot. 40:669-676.

12. Shiroya, T., G. R. Lister, V. Šlankis and G. Krotkov. 1966. Seasonal changes in respiration, photosynthesis, and translocation of the C labelled products of photosynthesis in young Pinus strobus L. plants. Annals of Bot. 30:81-89.

13. Srivastava, L. M. 1964. Anatomy, chemistry and physiology of bark. International Rev. For. Res. 1:203-277.

14. Stewart, C. M. 1966. The chemistry of secondary growth in trees. Division of Forest Products Tech. Paper No. 43. C.S.I.R.O. Melbourne, Australia.

15. Swanson, C. A. 1959. Translocation of organic solutes. In: Plant Physiology. A Treatise Vol. II. Ed., F. C. Steward. Academic Press. N. Y. pp. 481-551.

16. Trip, P., G. Krotkov and C. D. Nelson. 1964. Metabolism of mannitol in higher plants. Amer. J. Bot. 51:828-835.

17. Wachtendorf, W. 1964. Uber die Einsatzmoglichkeitin innertherapeutischer Praparate im Forstschutz. Anz. F. Schadlingskunde. 27:166-167.

18. Wardlaw, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.

19. Zimmermann, M. H. 1960. Transport in phloem. Ann. Rev. of Plant Physiol. 11:167-190.

# Chapter 3

## Photosynthetic Assimilation and Translocation of $^{3}H$- and $^{14}C$-Organic Compounds after $^{3}H_2O$ and $^{14}CO_2$ Were Simultaneously Offered to a Primary Leaf of Soybean

# INTRODUCTION

After a green leaf is placed in air containing tritiated water ($^3H_2O$), $^3H$ can be found in organic compounds and in water within the leaf. Some H bonds (O-H, N-H, S-H and Hal-H) readily exchange with water or alcohols. Others (C-H) are stable except in the presence of strong acids, strong bases or catalysts (14). Thus when $^3H_2O$ is fed to a plant, $^3H$ may be incorporated by enzymatic reduction reactions into non-exchangeable positions and it may also be incorporated by hydrogen-isotope exchange into exchangeable positions (8). Since most organic compounds contain some exchangeable hydrogens, physical exchange of $^3H_2O$ and organic compounds could result in uniform $^3H$ label in the ethanol-soluble organic compounds (14). The percentage of $^3H$ distributed in the exchangeable $^3H$ of the organic compounds indicates the relative amounts of these organic compounds in the extract but not necessarily the recent formation of these compounds in enzymatic reduction processes.

Photosynthetic assimilation of $^3H_2O$ and $^{14}CO_2$ has been compared in Chlorella (8). In the light, three times as much $^3H$ is incorporated into non-exchangeable positions as in the dark in Chlorella and the percentages of non-exchangeable $^3H$ distributed among organic compounds is similar to the percentages of $^{14}C$ distributed among organic compounds (8). Choi and Aronoff found that in soybean more $^3H$ is incorporated into organic compounds during photosynthesis than in the dark (4) but exchangeable $^3H$ was not separated from non-exchangeable $^3H$ so these results are not completely comparable to the Chlorella results.

In translocation experiments the water fraction has been labelled with $^{3}H_2O$ (1,2,4,11,16), the organic fraction with $^{3}H$-organic compounds formed during photosynthesis (1,4,7,16) and glucose-6-$^{3}H$ has been offered through a cut flap technique (15). In the above experiments using $^{3}H$-organic compounds formed during photosynthesis, no distinction was made between exchangeable $^{3}H$ and non-exchangeable $^{3}H$ in the organic compounds. Since exchangeable $^{3}H$ in organic compounds is in equilibrium with $^{3}H$ from water, it is not possible to determine the actual amounts of organic compounds and water translocated in these experiments.

In the following work the assimilation and translocation of non-exchangeable $^{3}H$-organic compounds were compared with the assimilation and translocation of $^{14}C$-organic compounds in young soybeans with the hope that both $^{3}H$- and $^{14}C$-labelled products of photosynthesis can be used in translocation experiments.

# MATERIALS AND METHODS

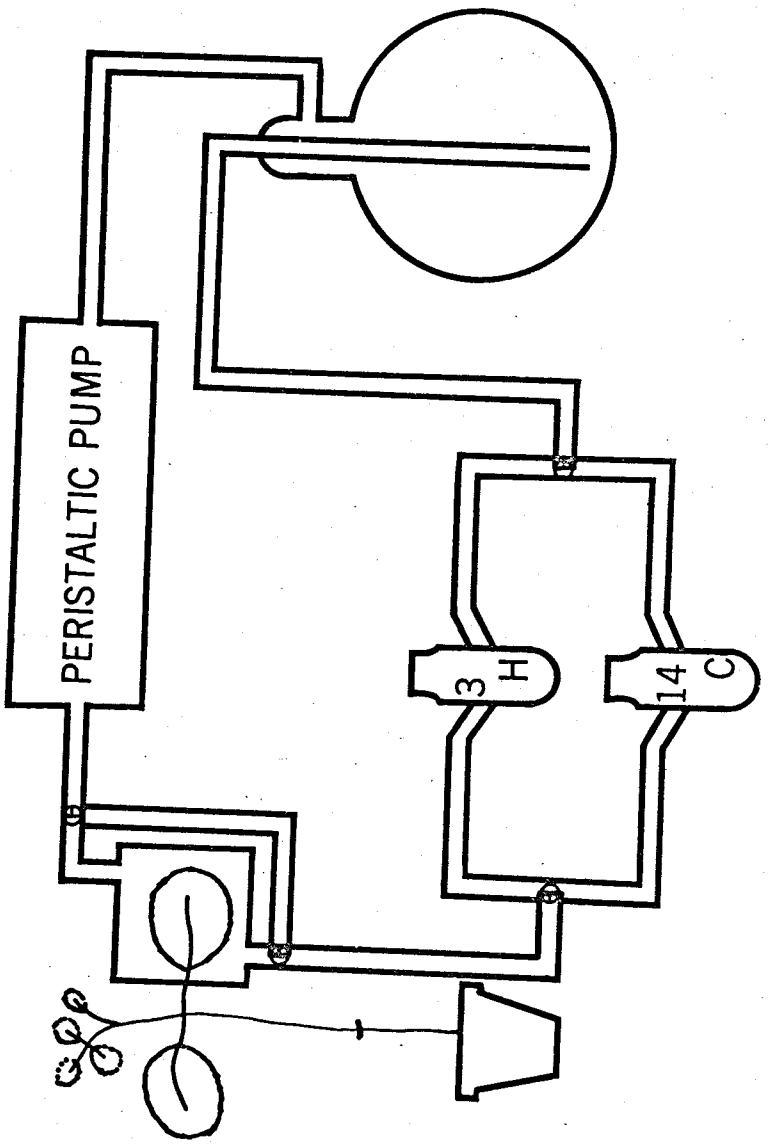
Soybeans, Glycine max L. var. Comet, were grown in soil in a greenhouse for 19 days. The plants were watered daily and fertilized with commercial fertilizer 20:20:20. They received approximately fourteen hours of daylight. Natural sunlight was supplemented with Grow-Lux fluorescent lights. A primary leaf on each plant was simultaneously offered $3.43 \times 10^{-8}$ moles of $^{3}H$ as $^{3}H_2O$ (50 mc) and $1.05 \times 10^{-8}$ moles of $^{14}CO_2$ (0.68 μc) in a closed-circuit feeding apparatus (Fig. 3.1). During the longer (30 minute) feedings a 2,000 ml flask was added to increase the volume of the circuit preventing the fed leaf from reaching the $CO_2$ compensation point.

After a primary leaf was placed in the chamber the plant was given a 30 minute pretreatment period. The leaf chamber was open to room air until just prior to feeding. Plants fed in the dark were kept in the dark during the pretreatment period. Plants fed in the light were illuminated by a Sylvania R30 Superflood Lamp ($3400^{\circ}K$) filtered through 10 cm of water to yield 2,000 ft-c measured with a Weston light meter.

Air was pumped through the closed circuit using the chamber by-pass and a Drierite column for 20 minutes to remove water vapour. Before generating $^{14}CO_2$ from $Na_2{}^{14}CO_3$ and 2N $H_2SO_4$ the Drierite column was removed from the circuit. $^{3}H_2O$ vapour was produced by heating the $^{3}H_2O$ generating flask and the gases were mixed by pumping them through the circuit for 5 minutes before the feeding began.

Two experimental plans were followed. In the first, the primary

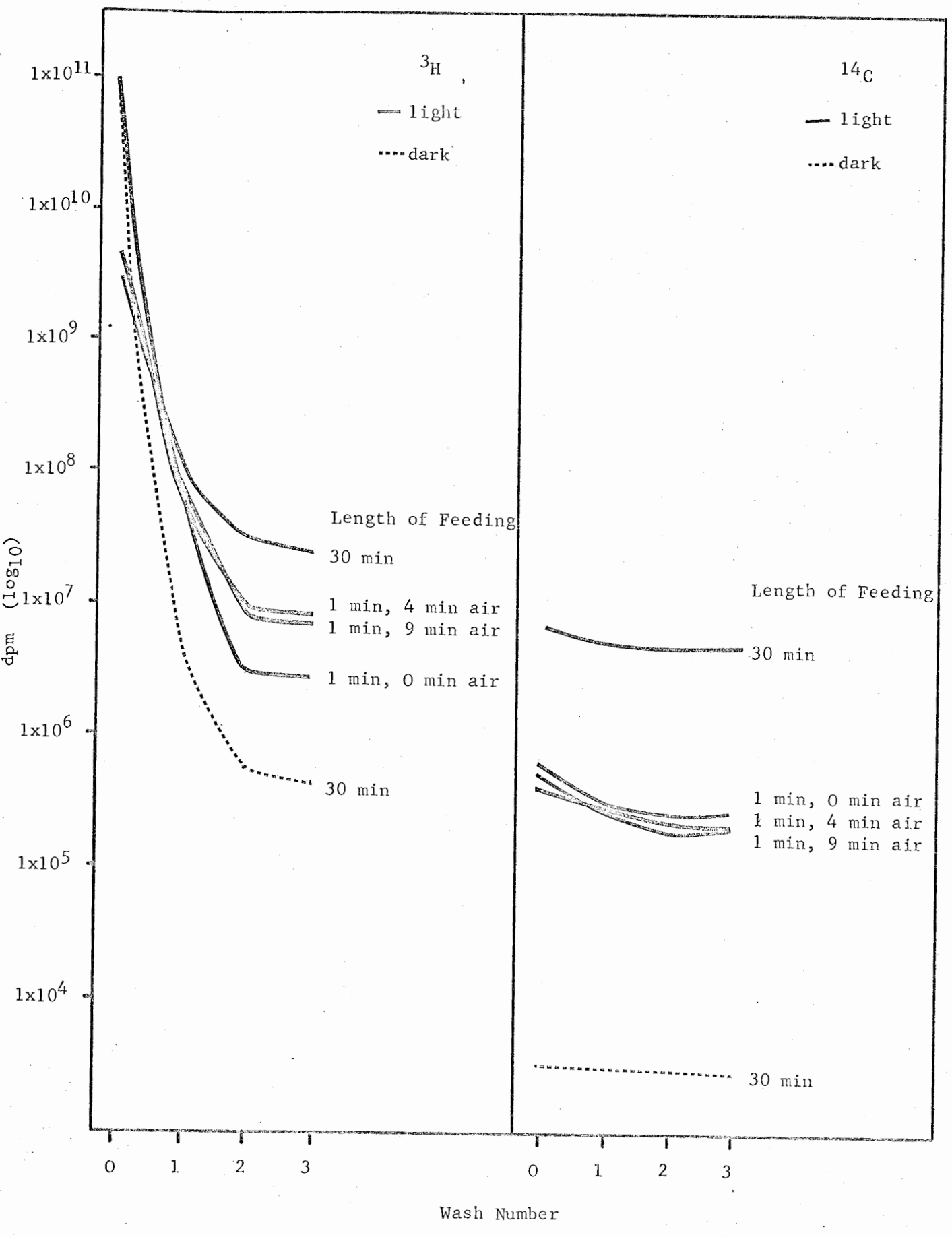
Figure 3.1 Apparatus for simultaneously feeding $^{3}H_2O$ and $^{14}CO_2$ to a primary leaf of soybean. Volume of circuit and chamber 350 ml. Ballast flask added 2,000 ml to circuit.


PERISTALTIC PUMP
3 H
14 C

leaves were fed $^{3}H_2O$ vapour and $^{14}CO_2$ for 1 minute followed by 0 minutes, 4 minutes or 9 minutes in air (300 ppm $CO_2$, 21% $O_2$) before the leaf was extracted with hot 80% ethanol : 20% water. Changes in percentage of non-exchangeable $^{3}H$ and $^{14}C$ distributed among the ethanol-soluble organic compounds was determined at the different times following feeding. In the second experimental plan the primary leaves were fed $^{3}H_2O$ vapour and $^{14}CO_2$ for 30 minutes in the light or in the dark. Before extraction with hot 80% ethanol the plants were cut into the following pieces: second trifoliate leaf, first trifoliate leaf, stem between first trifoliate leaf and primary leaf, fed primary leaf, opposite primary leaf, stem between the primary leaves and cotyledons, stem below cotyledons, and roots. Percentages of non-exchangeable $^{3}H$ and $^{14}C$ distributed among individual organic compounds were determined for the fed leaf and the stem between primary leaves and cotyledons. Total amounts of non-exchangeable $^{3}H$ and $^{14}C$ in the organic compounds distributed among the various pieces of the plant were also determined.

Each extract was evaporated to dryness, taken up in 50 ml of cold 80% ethanol and evaporated to dryness again. This process was repeated three times until $^{3}H$ dpm reached a steady value (Fig. 3.2) indicating that the easily exchangeable $^{3}H$ and $^{3}H_2O$ had been removed from the extract. Finally, the extracts were taken up in 1 ml of water and 1 ml of chloroform. When the chloroform phase had settled from the water (24 hrs), 100 µl aliquots of the water phase were assayed in a liquid scintillation spectrometer (Packard Tricarb Series 3000) for $^{3}H$ and $^{14}C$ using a dioxane-based scintillation solution. Corrections were made for counting efficiency with an

Figure 3.2 Loss of $^{3}H$ and $^{14}C$ from the ethanol-soluble extract with successive washings with 80% ethanol and evaporations to dryness.


$^3H$
light
dark
$^{14}C$
light
dark
$1x10^{11}$
$1x10^{10}$
$1x10^9$
$1x10^8$
$1x10^7$
$1x10^6$
$1x10^5$
$1x10^4$
dpm ($log_{10}$)
Length of Feeding
30 min
1 min, 4 min air
1 min, 9 min air
1 min, 0 min air
30 min
Length of Feeding
30 min
1 min, 0 min air
1 min, 4 min air
1 min, 9 min air
30 min
0
1
2
3
0
1
2
3
Wash Number

external standard (Radium 226). The chloroform fraction, which contained less than 1 percent of $^{3}H$ and $^{14}C$ in the total extract, was discarded.

For the 1 minute feedings the extracts from the fed leaf were separated into sugars, amino acids and organic acids using resin column chromatography—Rexyn 101 (H) and 201 (OH). Sugar, amino acids and organic acids fractions were separated into individual compounds using two dimensional paper chromatography, and identified with benzidine-TCA, ninhydrin and silver nitrate sprays respectively.

For the 30 minute feedings the sugars and amino acids in the extracts from the fed leaf and stem between primary leaves and cotyledons were separated using two dimensional paper chromatography. Sugar and amino acid spots were identified on replicate chromatograms using benzidine-TCA and ninhydrin sprays.

Organic compounds were eluted from chromatogram spots with distilled water running down a wick and dropping off the end of the chromatogram spot (5). Seven drops of water were found sufficient to remove 99% of the $^{3}H$ and $^{14}C$ from all chromatogram spots (Appendix 2). Amounts of $^{3}H$ and $^{14}C$ in the eluates were determined in a liquid scintillation spectrometer.

# RESULTS

## Assimilation of $^{3}H$ and $^{14}C$ into the ethanol-soluble organic compounds of the fed leaf.

Less than one percent of the $^{3}H$ offered to the primary leaf was assimilated into the non-exchangeable $^{3}H$ in the ethanol-soluble organic compounds in the light while from 10% to 36% of the $^{14}C$ was assimilated into the ethanol-soluble organic compounds (Table 3.1). In all experiments there was more variation in assimilation of $^{3}H$ than $^{14}C$. After 4 minutes following a 1 minute feeding, about 20 times more $^{3}H$ was assimilated than immediately after the 1 minute feeding (Table 3.1). There was no increase in assimilation of $^{3}H$ between 4 and 9 minutes after the 1 minute feeding. Assimilation of $^{14}C$ into organic compounds did not increase with time from the end of feeding.

In the 30 minute feedings the assimilation of non-exchangeable $^{3}H$ into the ethanol-soluble fraction in the dark was about ten percent of the assimilation of $^{3}H$ in the light (Table 3.1). In a similar experiment with soybean, Choi and Aronoff also measured the assimilation in the dark to be about ten percent of the assimilation in the light (4). Assimilation of $^{14}C$ into the ethanol-soluble organic compounds in the dark was less than one percent of the assimilation of $^{14}C$ in the light (Table 3.1). Sen and Leopold showed for soybean that the assimilation of $^{14}C$ in the dark was about 0.5% of the assimilation in the light (13).

Although the 30 minute feedings lasted 30 times longer only about three times as much $^{3}H$ and $^{14}C$ were incorporated as in 1 minute feedings

Table 3.1 Assimilation of non-exchangeable $^{3}H$ and $^{14}C$ into the ethanol-soluble organic compounds of primary leaves of 19-day-old soybeans offered $3.43 \times 10^{-8}$ moles (50 mc) of $^{3}H$ as $^{3}H_2O$ vapour and $1.05 \times 10^{-8}$ moles (0.68 μc) of $^{14}C$ as $^{14}CO_2$ simultaneously.

| experimental conditions | moles of isotope assimilated x $10^{-12}$ | | isotope assimilated as a percent of isotope offered. | |
|---|---|---|---|---|
| | $^{3}H$ | $^{14}C$ | $^{3}H$ | $^{14}C$ |
| 1 minute feeding, 0 minutes in air, in the light. | 6.74 ± 56%[1.] | 1890 ± 28%[1.] | .02% | 18% |
| 1 minute feeding, 4 minutes in air, in the light. | 118 ± 49% | 1400 ± 23% | .34% | 13% |
| 1 minute feeding, 9 minutes in air, in the light. | 101 ± 70% | 1520 ± 45% | .29% | 14% |
| 30 minute feeding in the light. | 328 ± 62% | 3850 ± 38% | .96% | 37% |
| 30 minute feeding in the dark. | 38.8 ± 74% | 20.2 ± 71% | .11% | 0.2% |

1. each value is the mean of four plants ± coefficient of variation.

followed by 9 minutes in air (Table 3.1). In the 30 minute feedings, the total amount of $^{3}H_2O$ vapour and $^{14}CO_2$ offered was the same but the concentration of the radioisotopes was decreased as the volume of the closed circuit was 2,000 ml larger than in the 1 minute feedings.

Metabolism of non-exchangeable $^{3}H$- and $^{14}C$-organic compounds in the fed leaf.

Changes in percentages of non-exchangeable $^{3}H$ closely paralleled changes in percentages of $^{14}C$ distributed in the sugars, amino acids and organic acids at 0, 4 and 9 minutes following a 1 minute feeding (Fig. 3.3). There was no difference at the 95% significance level between the individual values for $^{3}H$ and $^{14}C$. Percentages of $^{14}C$ distributed among the ethanol-soluble compounds of the primary leaves after 1 minute followed by 9 minutes in air were typical of the results obtained for 19-day-old soybeans after 10 minutes photosynthesis with $^{14}CO_2$ (9).

Percentages of non-exchangeable $^{3}H$ distributed in individual sugars were similar to the distribution of $^{14}C$ (Fig. 3.4). About 70%-80% of $^{3}H$ and $^{14}C$ in the sugars was found in sucrose, about 15% in glucose, 5%-10% in fructose and trace amounts in other sugars (Fig. 3.4). These percentages of $^{14}C$ were typical of other experiments with soybean where $^{14}C$ is assimilated photosynthetically from $^{14}CO_2$ for 20 minutes (18). Percentages of non-exchangeable $^{3}H$ distributed in aspartic acid, asparagine and glutamine were similar to the distribution of $^{14}C$ (Fig. 3.5). Glutamic acid contained a greater percentage of $^{3}H$ than $^{14}C$ (79% : 4.9%) for 1 minute feedings followed by 0 minutes in air. Differences between the percentage of the $^{3}H$ and $^{14}C$ decreased after 4 minutes and 9 minutes in air but remain significant. Glycine-serine has the opposite pattern,

Figure 3.3 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in the sugars, amino acids and organic acids after 1 minute photosynthesis with $^{3}H_2O$ and $^{14}CO_2$ followed by 0, 4, 9 minutes in air. Each value is the mean for 4 primary leaves of soybean ± standard deviation.

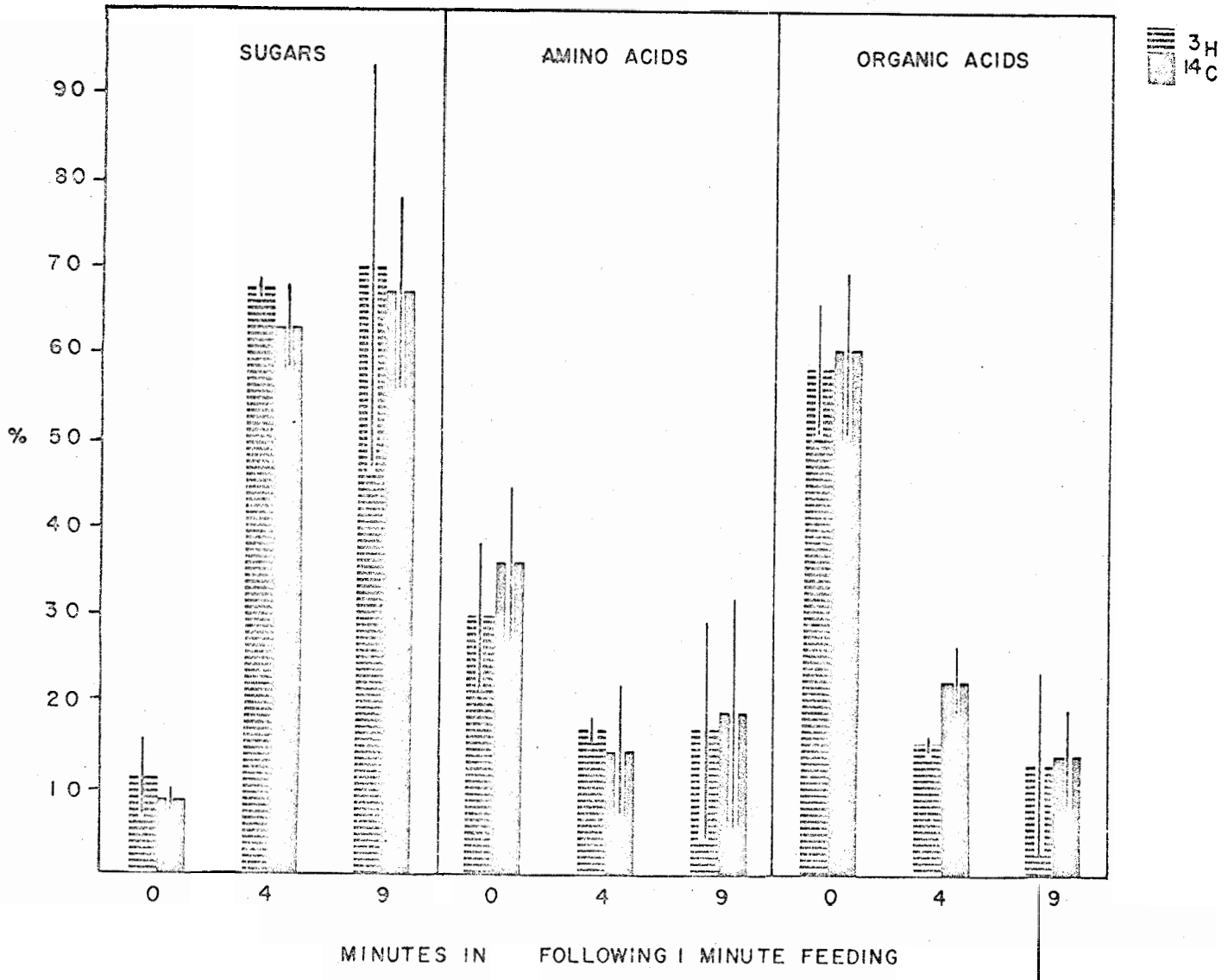

SUGARS
AMINO ACIDS
ORGANIC ACIDS
3H
14C
90
80
70
60
%
50
40
30
20
10
0
4
9
0
4
9
0
4
9
MINUTES IN FOLLOWING I MINUTE FEEDING

Figure 3.4 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in individual sugars expressed as a percentage of total sugars after 1 minute photosynthesis with $^{3}H_2O$ and $^{14}CO_2$ followed by 0, 4 and 9 minutes in air. Each value is the mean for 4 primary leaves of soybean ± standard deviation.

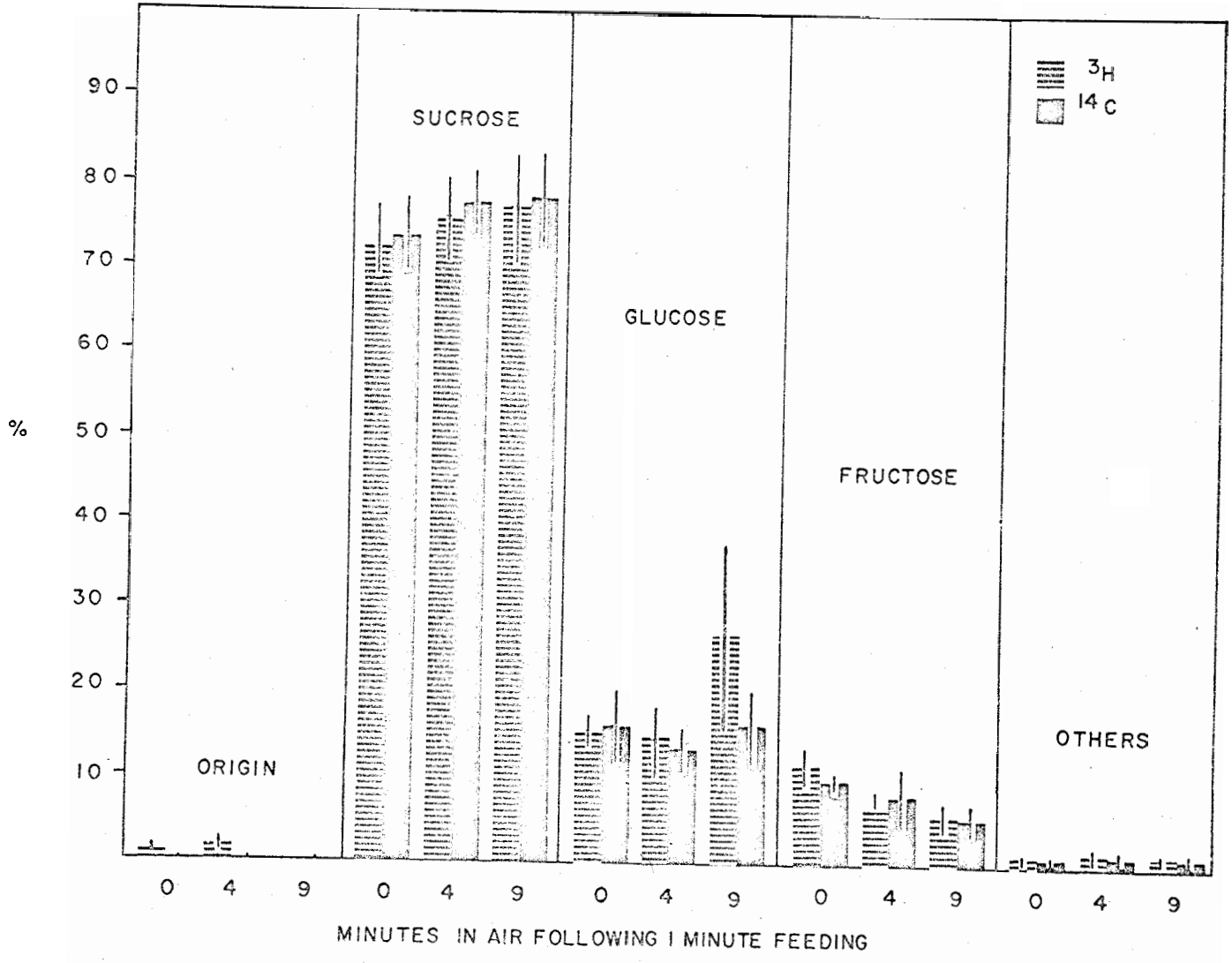
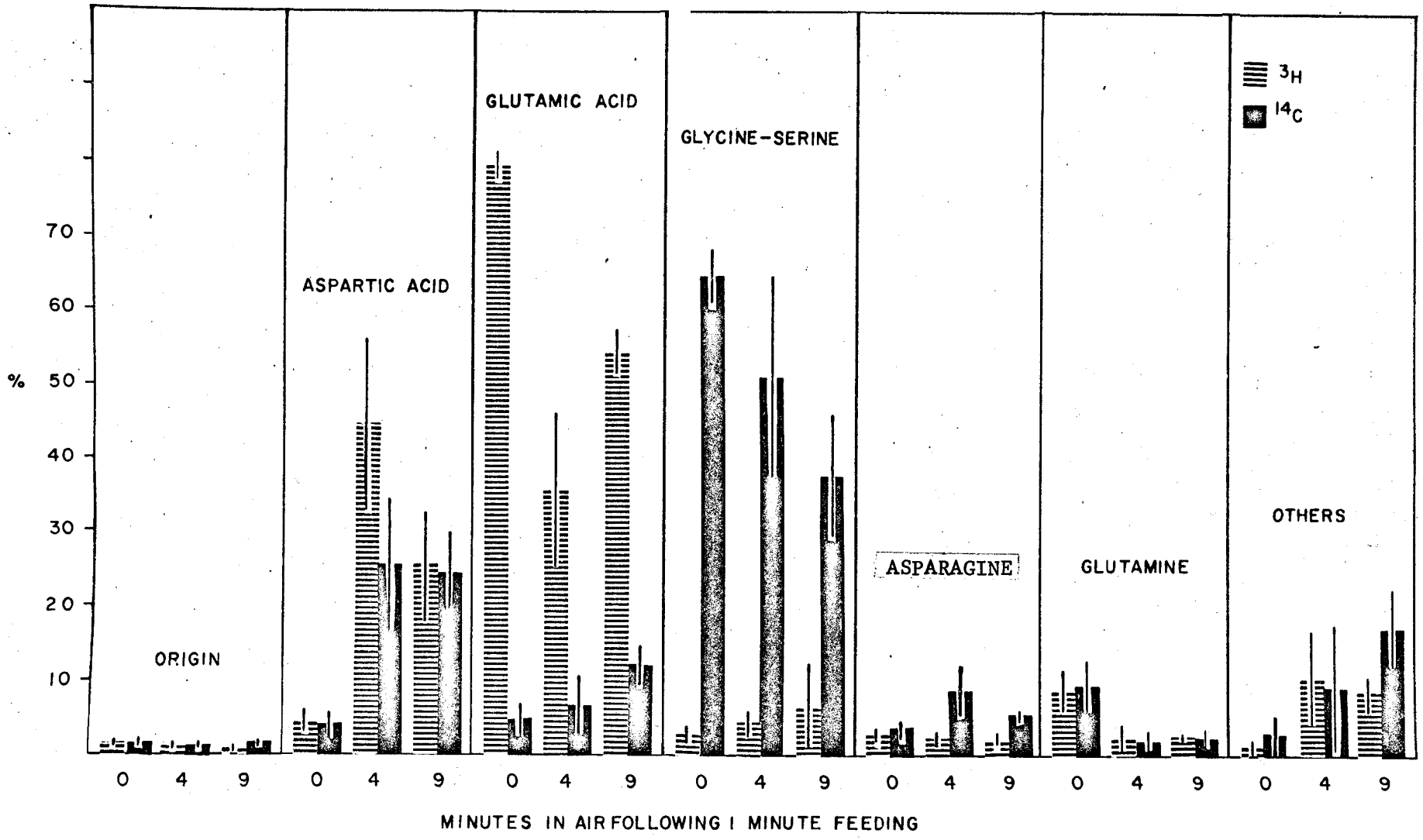

90
80
70
60
%
50
40
30
20
10
SUCROSE
GLUCOSE
FRUCTOSE
OTHERS
ORIGIN
$^{3}H$
$^{14}C$
0 4 9 0 4 9 0 4 9 0 4 9 0 4 9
MINUTES IN AIR FOLLOWING I MINUTE FEEDING

Figure 3.5 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in individual amino acids after 1 minute photosynthesis with $^{3}H_2O$ and $^{14}CO_2$ followed by 0, 4 and 9 minutes in air. Each value is the mean of 4 primary leaves of soybean ± standard deviation.


ORIGIN
ASPARTIC ACID
GLUTAMIC ACID
GLYCINE-SERINE
ASPARAGINE
GLUTAMINE
OTHERS
$^{3}H$
$^{14}C$
%
70
60
50
40
30
20
10
0 4 9 0 4 9 0 4 9 0 4 9 0 4 9 0 4 9 0 4 9
MINUTES IN AIR FOLLOWING 1 MINUTE FEEDING

but there was a significantly greater percentage of $^{14}C$ than $^{3}H$ (75% : 2.6%) for the 1 minute feeding followed by 0 minutes in air. Differences between $^{3}H$ and $^{14}C$ in glycine-serine decreased with a longer time for metabolic turnover. Comparable differences between glutamic acid and serine labelling patterns are also found in Chlorella (8). Distribution of $^{14}C$ in the amino acids was similar to the distribution of $^{14}C$ in other experiments with soybean (12).

Percentages of $^{3}H$ distributed in individual organic acids for the 1 minute feedings were about the same as that of $^{14}C$ distributed in individual organic acids (Fig. 3.6). Since there was more $^{3}H$ in the organic acids in 1 minute feedings followed by 0 minutes in air (Fig. 3.3) it was easier to detect a greater number of $^{3}H$-organic compounds.

Translocation of non-exchangeable $^{3}H$- and $^{14}C$-organic compounds.

After a 30 minute simultaneous feeding of $^{3}H_2O$ and $^{14}CO_2$ both non-exchangeable $^{3}H$ and $^{14}C$ were translocated in the light (Fig. 3.7). Percentages of $^{3}H$ and $^{14}C$ of total translocated were similar in various parts of the plant. In the dark there was no translocation of $^{14}C$ but percentages of $^{3}H$ distributed in different parts of the plant were similar to that found in the light.

In the fed leaf in the light after a 30 minute feeding the percentages of non-exchangeable $^{3}H$ distributed in the ethanol-soluble organic compounds were similar to that of $^{14}C$ (Fig. 3.8). At least 60% of $^{3}H$ and $^{14}C$ was found in sugars (sucrose, glucose and fructose). Large amounts of $^{3}H$ and $^{14}C$ in sucrose and smaller amounts in glucose, fructose, amino acids and organic acids in the 30 minute feedings were similar to the distribution

Figure 3.6 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in individual organic acids after 1 minute photosynthesis with $^{3}H_2O$ and $^{14}CO_2$ followed by 0, 4 and 9 minutes in air. Each value is the mean of 4 primary leaves of soybean ± standard deviation.

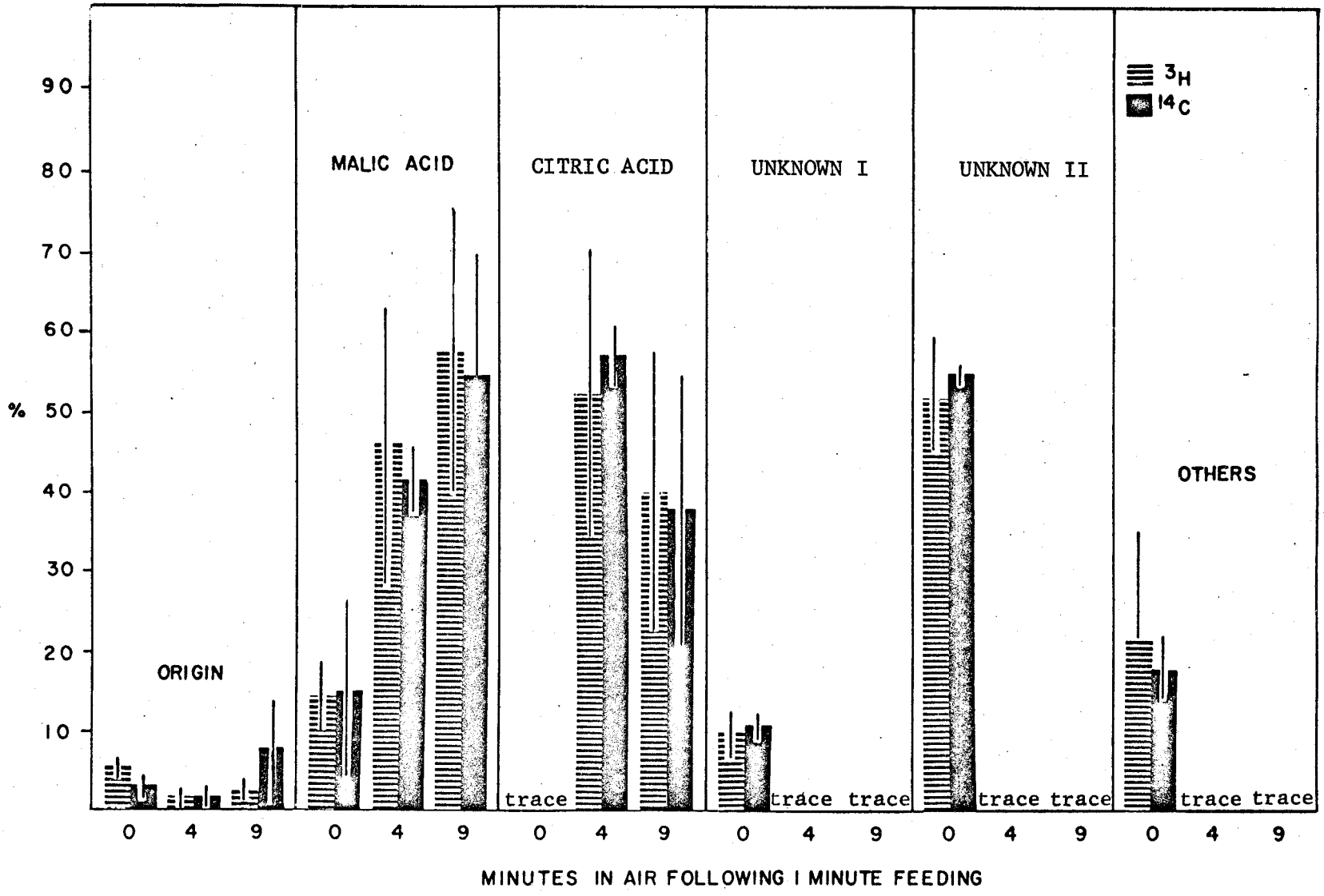

90
80
70
60
%
50
40
30
20
10
ORIGIN
MALIC ACID
CITRIC ACID
UNKNOWN I
UNKNOWN II
OTHERS
3H
14C
trace
trace trace
trace trace
trace trace
0 4 9 0 4 9 0 4 9 0 4 9 0 4 9 0 4 9
MINUTES IN AIR FOLLOWING I MINUTE FEEDING

Figure 3.7 Translocation of non-exchangeable $^{3}H$ and of $^{14}C$ in the ethanol-soluble organic compounds after a 30 minute feeding with $^{3}H_2O$ and $^{14}CO_2$ both in the light and in the dark. Percentage translocation in each plant piece is expressed as a percentage of total $^{3}H$ and $^{14}C$ translocated. Each value is the mean of 4 soybeans ± standard deviation.

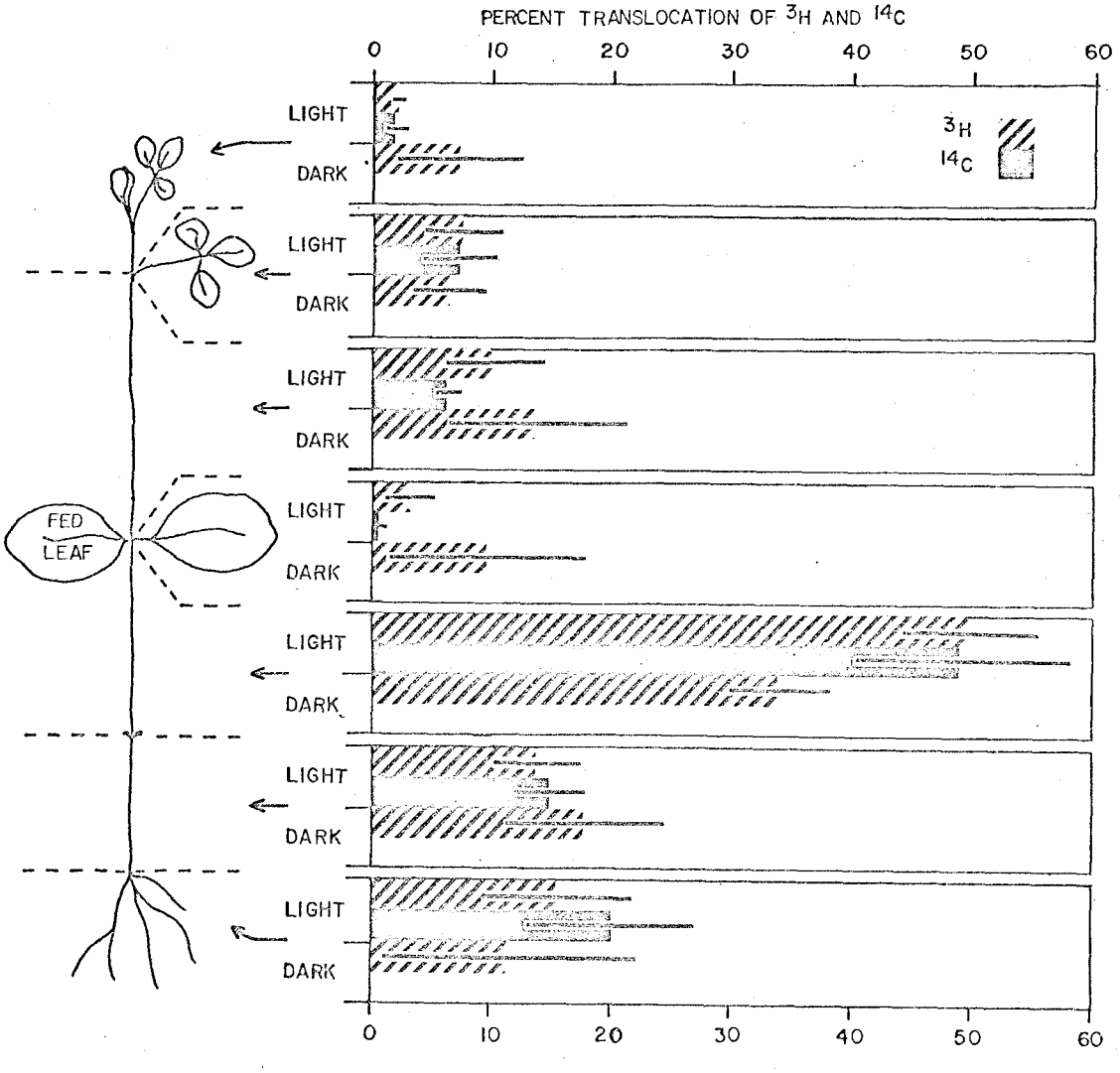
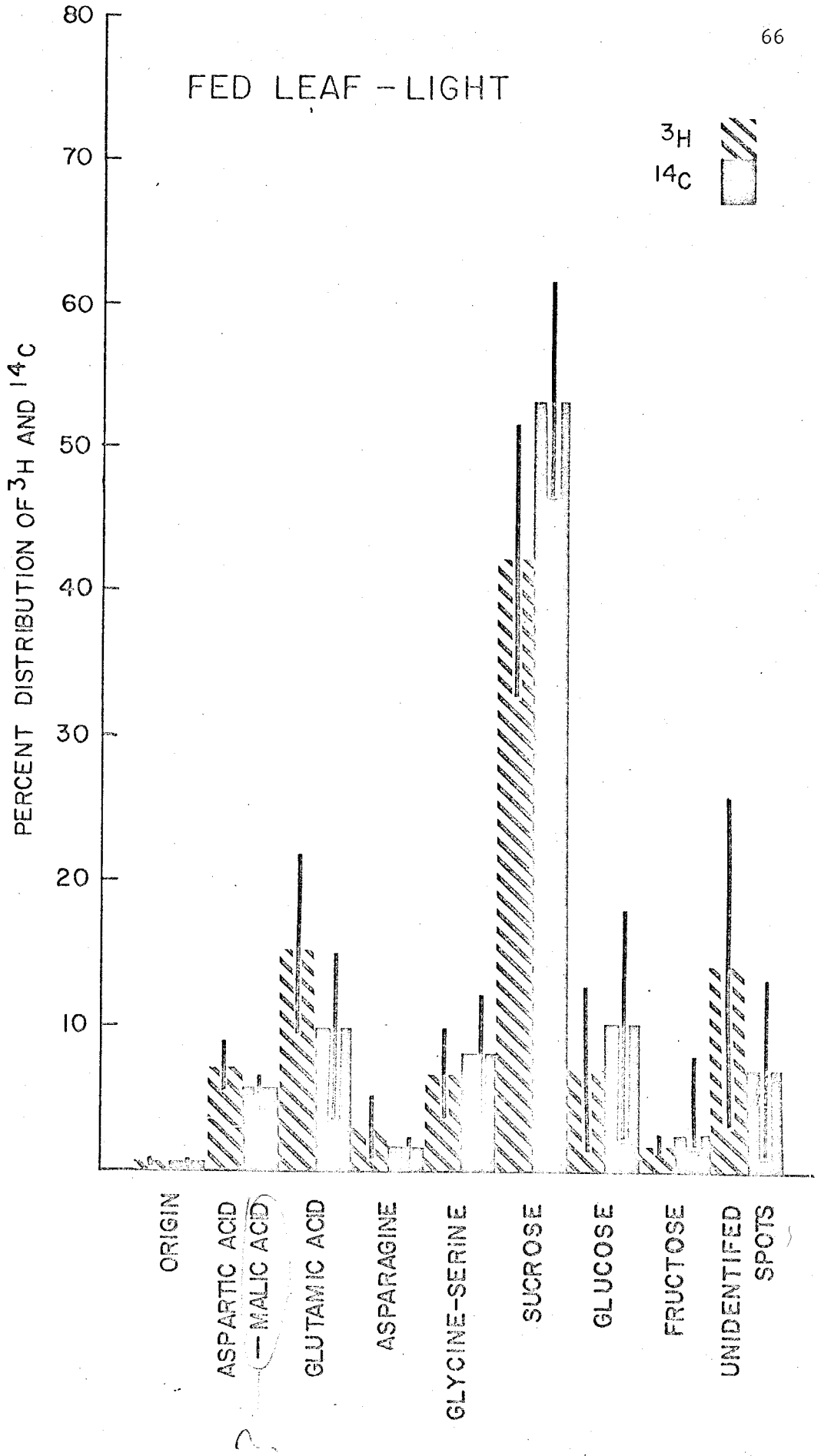

PERCENT TRANSLOCATION OF $^{3}H$ AND $^{14}C$
0
10
20
30
40
50
60
LIGHT
DARK
$^{3}H$
$^{14}C$
FED
LEAF

Figure 3.8 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in individual organic compounds of the fed leaf expressed as a percentage of total $^{3}H$ or $^{14}C$ in the organic compounds after 30 minutes photosynthesis with $^{3}H_2O$ and $^{14}CO_2$. Each value is the mean of 4 soybeans ± standard deviation.


FED LEAF – LIGHT
$^3H$
$^{14}C$
PERCENT DISTRIBUTION OF $^3H$ AND $^{14}C$
80
70
60
50
40
30
20
10
ORIGIN
ASPARTIC ACID
MALIC ACID
GLUTAMIC ACID
ASPARAGINE
GLYCINE-SERINE
SUCROSE
GLUCOSE
FRUCTOSE
UNIDENTIFED SPOTS

of $^{3}H$ and $^{14}C$ in the 1 minute feedings after 4 and 9 minutes in air.

In the stem in the light after a 30 minute feeding, the percentages of non-exchangeable $^{3}H$ distributed in the ethanol-soluble organic compounds were the same as the percentages of $^{14}C$ (Fig. 3.9). The distribution of the $^{14}C$ in the light was typical of distributions of $^{14}C$ after 30 minutes (9).

Dark assimilation of almost sixty percent of the total $^{14}C$ into aspartic acid-malic acid of the fed leaf (Fig. 3.10) is typical of $^{14}C$ assimilation into organic compounds (10,12). Assimilation of $^{3}H$ into the ethanol-soluble organic compounds in the dark was not the same as the assimilation of $^{14}C$ in the dark. There was more $^{3}H$ than $^{14}C$ in aspartic acid-malic acid. Sugars contained some non-exchangeable $^{3}H$ but no $^{14}C$.

In the dark the percentage of non-exchangeable $^{3}H$ distributed in the organic compounds of the stem was similar to the distribution pattern in the fed leaf (Table 3.2).

No $^{14}C$ was found in the ethanol-soluble organic compounds of the stem of plants fed in the dark (Fig. 3.11). This is expected, since $^{14}C$ is translocated as sucrose and no $^{14}C$ was assimilated into sucrose in the fed leaf in the dark.

Figure 3.9 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in individual organic compounds of the stem expressed as a percentage of total $^{3}H$ or $^{14}C$ in the organic compounds after 30 minutes photosynthesis with $^{3}H_2O$ and $^{14}CO_2$. Each value is the mean for 4 soybeans ± standard deviation.

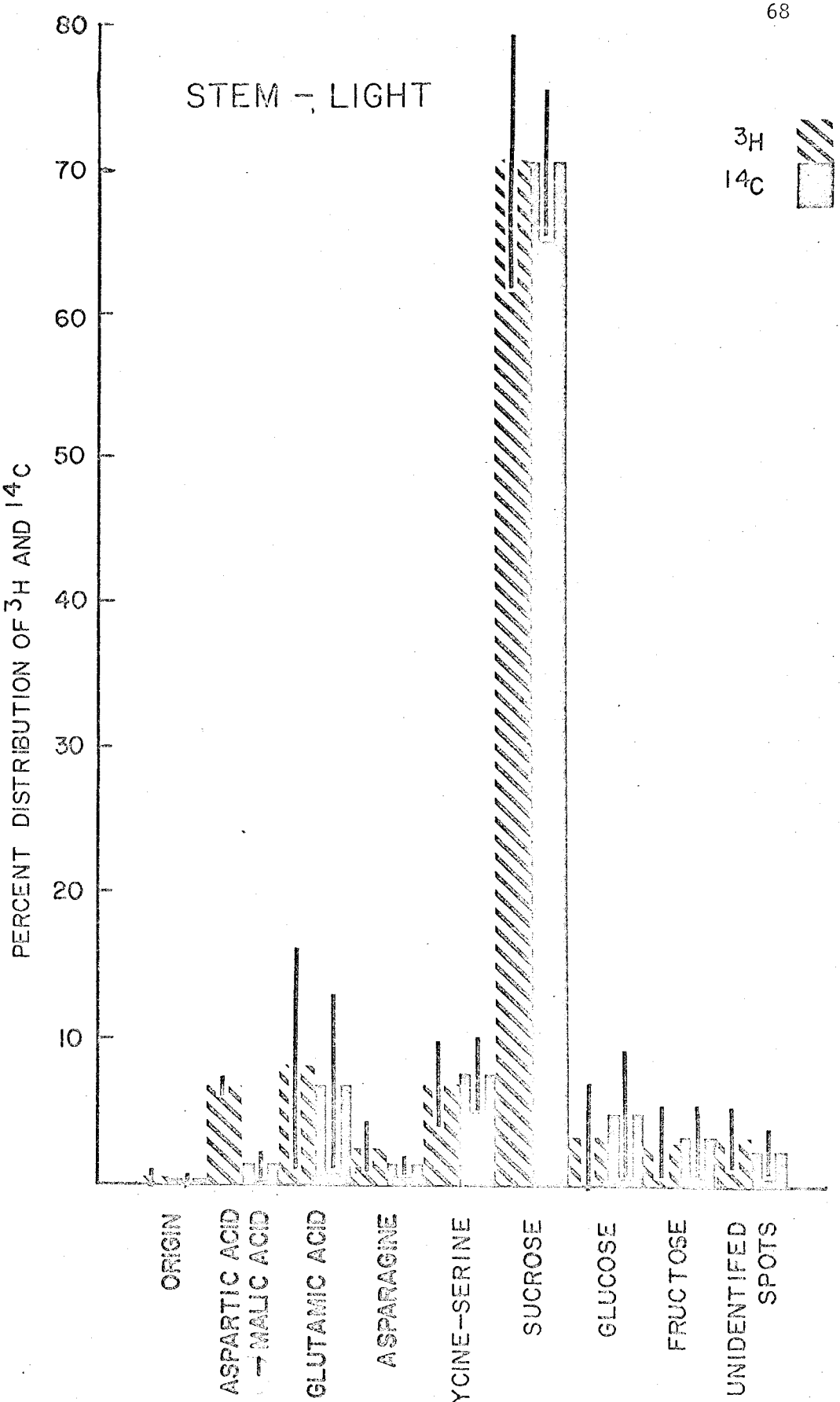

STEM – LIGHT
$^3H$
$^{14}C$
PERCENT DISTRIBUTION OF $^3H$ AND $^{14}C$
80
70
60
50
40
30
20
10
ORIGIN
ASPARTIC ACID – MALIC ACID
GLUTAMIC ACID
ASPARAGINE
GLYCINE–SERINE
SUCROSE
GLUCOSE
FRUCTOSE
UNIDENTIFED SPOTS

Figure 3.10 Percentage of non-exchangeable $^{3}H$ and of $^{14}C$ distributed in individual organic compounds of the fed leaf expressed as a percentage of total $^{3}H$ and $^{14}C$ in the organic compounds after 30 minutes dark assimilation with $^{3}H_2O$ and $^{14}CO_2$. Each value is the mean of 4 soybeans ± standard deviation.

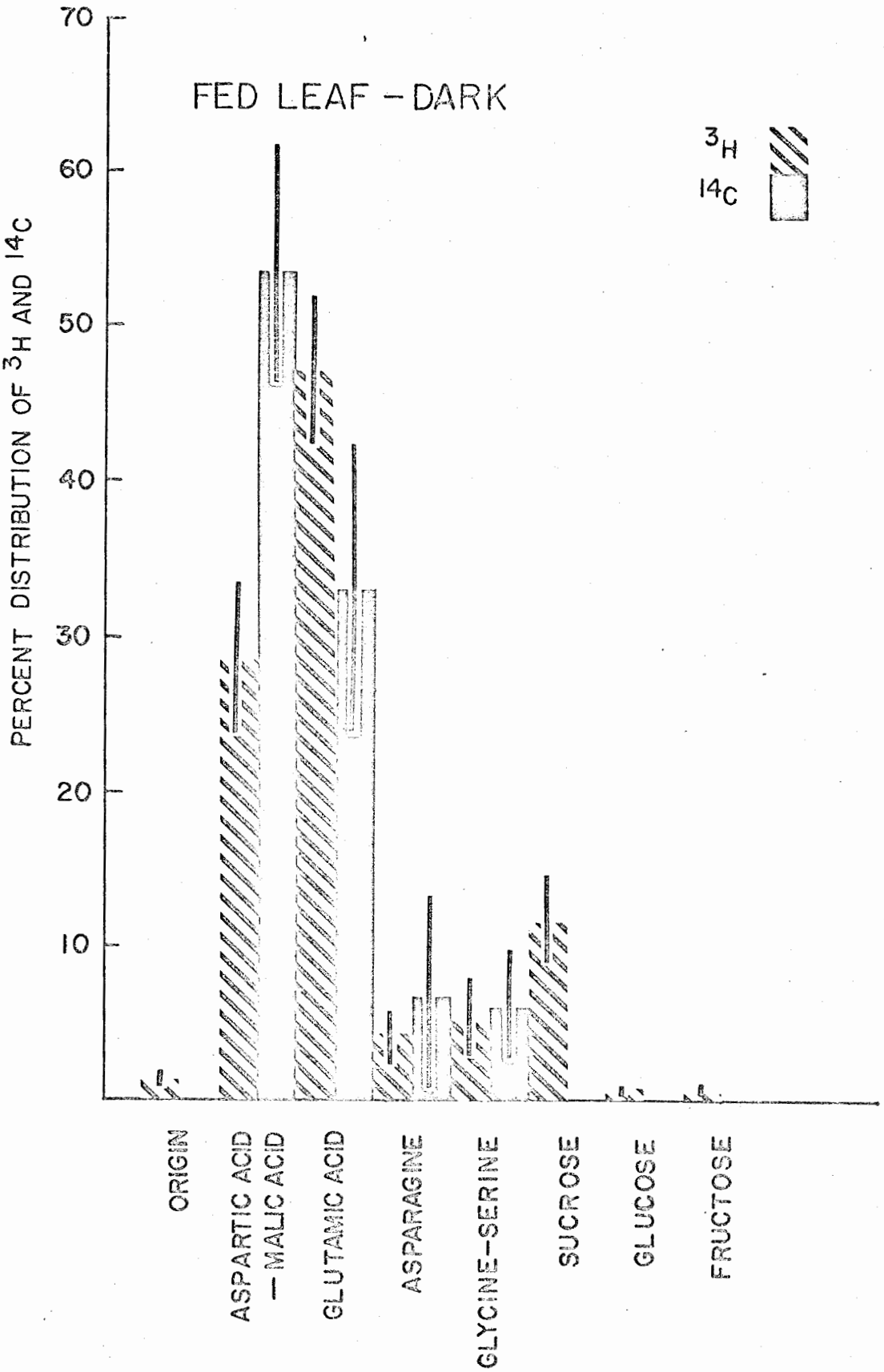
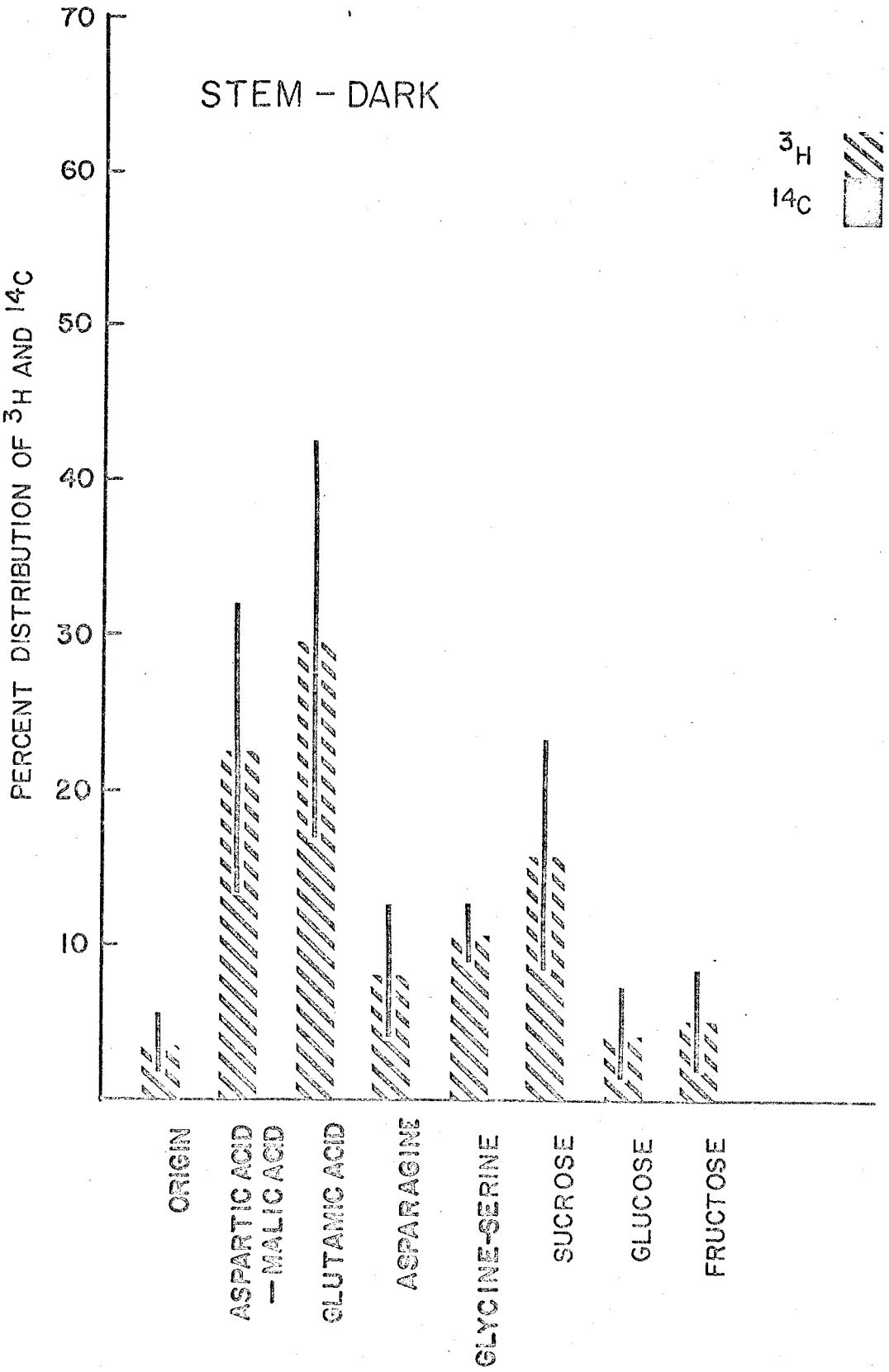

FED LEAF – DARK
$^{3}H$
$^{14}C$
PERCENT DISTRIBUTION OF $^{3}H$ AND $^{14}C$
70
60
50
40
30
20
10
ORIGIN
ASPARTIC ACID
— MALIC ACID
GLUTAMIC ACID
ASPARAGINE
GLYCINE–SERINE
SUCROSE
GLUCOSE
FRUCTOSE

Table 3.2. Distribution of $^{3}H$ in the ethanol-soluble organic compounds of 19-day-old soybeans fed in the dark.

| | [1] amount of $^{3}H$ found in each chromatogram spot expressed as a percent of the total activity found on the chromatogram. | |
|---|---|---|
| | fed leaf | stem |
| origin | 1.5 ± 27% | 3.7 ± 54% |
| malic acid-aspartic acid | 28.6 ± 17% | 22.6 ± 42% |
| glutamic acid | 47.1 ± 11% | 29.6 ± 43% |
| asparagine | 4.6 ± 28% | 8.2 ± 52% |
| glycine-serine | 5.4 ± 48% | 10.7 ± 17% |
| sucrose | 11.7 ± 24% | 15.8 ± 48% |
| glucose | 0.7 ± 42% | 4.3 ± 72% |
| fructose | 0.5 ± 60% | 5.2 ± 63% |

1. each value is the mean of four plants ± the coefficient of variation.

Figure 3.11 Percentage of non-exchangeable $^{3}H$ distributed in individual organic compounds of the stem expressed as a percentage of total $^{3}H$ in the organic compounds after 30 minutes dark assimilation. Each value is the mean of 4 soybeans ± standard deviation.


STEM – DARK
$^3H$
$^{14}C$
PERCENT DISTRIBUTION OF $^3H$ AND $^{14}C$
70
60
50
40
30
20
10
ORIGIN
ASPARTIC ACID —MALIC ACID
GLUTAMIC ACID
ASPARAGINE
GLYCINE-SERINE
SUCROSE
GLUCOSE
FRUCTOSE

# DISCUSSION

Analysis of the ethanol-soluble compounds both in the fed leaf and the remainder of the plant indicates that the distribution of non-exchangeable $^{3}H$ in different regions of the plant and in individual organic compounds is comparable to the distribution of $^{14}C$. However there are some differences. Assimilation of $^{3}H$ of the total $^{3}H_2O$ offered was less and had a greater variation compared to the assimilation of $^{14}C$ of the total $^{14}CO_2$ offered. The fed leaf continued to assimilate non-exchangeable $^{3}H$ into organic compounds after the 1 minute feeding stopped but did not assimilate more $^{14}C$. There was a difference in distribution of $^{3}H$ and $^{14}C$ in glutamic acid and glycine-serine.

Less assimilation and greater variation of $^{3}H$ compared with $^{14}C$ results from a combination of several factors, including the dilution of $^{3}H_2O$ by water already present in the fed leaf, the dilution of $^{3}H_2O$ by water from the roots, the loss of $^{3}H_2O$ from the fed leaf to the translocation stream, the loss of exchangeable $^{3}H$ in the organic compounds and the difficulty of getting all the water offered to the plant into the vapour form in the closed circuit of the feeding apparatus.

Continued assimilation of $^{3}H_2O$ after the 1 minute feeding had stopped can be accounted for by a larger pool of water in the leaf. This pool is difficult to flush and $^{3}H_2O$ remains in the leaf and continues to be assimilated after the leaf is no longer surrounded with $^{3}H_2O$ vapour. The smaller pool of $^{14}CO_2$ in the leaf is more easily flushed and the $^{14}C$ feeding is a better "pulse-chase" experiment than the $^{3}H$ feeding.

One might expect some $^{3}H$ label is formed by exchange of easily exchangeable hydrogen followed by metabolism into non-exchangeable positions. That this process does not account for a large part of the non-exchangeable $^{3}H$ label in soybean is indicated by the small amount of $^{3}H$ in sucrose in the dark (Fig. 3.10). Since the concentration of endogenous sugars in the leaf is far larger than any of the other compounds the sugars would be expected to contain the bulk of the $^{3}H$ if physical exchange were the main process of labelling in the dark. Thus, it is more likely that the bulk of the $^{3}H$ in the ethanol-soluble fractions occurs by enzymatic fixation rather than physical exchange.

The relatively low amount of label in sucrose and malic acids are neither the typical patterns obtained with $^{14}C$ in photosynthetic fixation or in dark fixation. It is known that only a few organic compounds are translocated from leaves (18,19). Previous work has shown (9) that small amounts of $^{14}C$ in compounds other than sucrose are derived from conversion of the translocated sucrose rather than the translocation of the non-sucrose compounds from the primary leaf. The similar distribution of $^{3}H$ in leaf and stem in the dark suggests that the compounds isolated from the stem were not translocated as such from the assimilating leaf but that $^{3}H_2O$ was the form in which $^{3}H$ was translocated giving rise after translocation to the several compounds isolated from the stem.

If such a mechanism for translocation of $^{3}H$ exists in the dark it is probable that it also exists in the light. However, the assimilation of $^{3}H$ in the dark was only at best one-tenth of that in the light (Table 2.1). Any translocation of water in the light and subsequent dark assimilation

into non-exchangeable $^{3}H$ in organic compounds could be masked by the large amount of $^{3}H$-sucrose translocated in the light. This is evident, since in the light the distribution of non-exchangeable $^{3}H$ in the organic compounds of the stem (Fig. 3.9) does not resemble the distribution pattern of $^{3}H$ and $^{14}C$ in the fed leaf (Fig. 3.8) but resembles the distribution pattern of $^{14}C$ in the stem (Fig. 3.9).

Distribution of non-exchangeable $^{3}H$ and $^{14}C$ in the ethanol-soluble organic compounds of the fed leaf for the 30 minute feeding (Fig. 3.8) was consistent with the result of the 1 minute feedings (Fig. 3.4, 3.5, 3.6). At least 60% of non-exchangeable $^{3}H$ and $^{14}C$ was found in sugar for the 30 minute feedings compared to about 70% in sugar for the 1 minute feedings followed by 9 minutes in air and about 10% in sugar for the 1 minute feeding followed by 0 minutes in air (Fig. 3.3). In the 30 minute feedings non-exchangeable $^{3}H$ and $^{14}C$ in sugar was slightly less than in the 1 minute feeding left for 9 minutes in air since the percentage of $^{3}H$ and $^{14}C$ distributed in the sugars was always affected by recently assimilated $^{3}H$ and $^{14}C$ into the sugars. However, in the 1 minute feedings followed by 9 minutes in air, the percentage of $^{3}H$ and $^{14}C$ distributed in the sugars results from metabolic turnover and was not altered by recently assimilated $^{3}H$ and $^{14}C$ into the sugars. Lower percentages of $^{3}H$ with greater percentages of $^{14}C$ in glycine-serine and the reverse in glutamic acid was seen in 30 minute feedings as well as 1 minute feedings. However the difference for the 30 minute feedings are not statistically significant. Decreased statistical significance of the 30 minute feedings as compared to the 1 minute feedings is consistent with the longer time for metabolic turnover

in the 30 minute feedings which allows the initial difference to be masked.

The difference between the distribution of non-exchangeable $^{3}H$ and $^{14}C$ in glycine-serine and glutamic acid leads to an interesting speculation. If glycine and serine contain very recently incorporated carbon then they might be expected to be high in $^{14}C$ (17). If the chloroplast, the region of carbon assimilation from $CO_2$, is isolated by a membrane and not freely interconnected with water outside the chloroplast then the organic compounds recently formed in photosynthesis would be low in $^{3}H$. Glycine-serine is low in non-exchangeable $^{3}H$ and high in $^{14}C$. If non-exchangeable $^{3}H$ is incorporated into ensuing products of photosynthesis by enzymatic reduction, perhaps outside the chloroplast in regions freely mixed with $^{3}H_2O$ entering the cell, then non-exchangeable $^{3}H$ is not a direct indication of photosynthetic assimilation but is a measure of the metabolic activity of the organic compounds in which it is assimilated. More metabolically active compounds assimilate more non-exchangeable $^{3}H$ than less metabolically active compounds. Compounds involved in "dark fixation" in photosynthesis are more metabolically active and have assimilated more non-exchangeable $^{3}H$. Since more non-exchangeable $^{3}H$ is assimilated in the light than in the dark this would mean that soybean is more metabolically active in the light than in the dark. The increased rate of "photorespiration" over "dark respiration" suggests soybeans in the light are ten times more metabolically active (2,6), which corresponds to ten times more $^{3}H$ fixation in the light than in the dark.

The large percentage of non-exchangeable $^{3}H$ in glutamic acid, between

40 to 80% for the 1 minute feedings, indicates that glutamic acid is a metabolically active compound in soybean. The low percentage of $^{14}C$ in glutamic acid indicates the precursors to glutamic acid are a number of metabolic steps removed from the initial carbon incorporation step (17).

The ten-fold increase of $^{3}H$ and the hundred-fold increase of $^{14}C$ in the fed leaf of plants in the light over plants in the dark, indicates that the major process in the assimilation of non-exchangeable $^{3}H$ and $^{14}C$ into organic compounds is photosynthetic assimilation. Comparable percentages of $^{3}H$ and $^{14}C$ distributed in the organic compounds of the assimilation leaf of plants in the light, indicates that photosynthetic assimilation involves similar organic compounds for both carbon and hydrogen. The compounds active in $CO_2$ assimilation in photosynthesis are more metabolically active and assimilate more hydrogen.

In summary, the simultaneous assimilation and translocation of $^{3}H$ and $^{14}C$ both at the level of the quantities of total isotope translocated and the level of the distribution of isotope in the organic compounds indicates that:

1. photosynthesis is the most important process in the assimilation of non-exchangeable $^{3}H$ and $^{14}C$ into organic compounds in the light.

2. non-photosynthetic assimilation is more important for non-exchangeable $^{3}H$ than $^{14}C$.

3. $^{3}H$ is translocated as $^{3}H_2O$ as well as non-exchangeable $^{3}H$ in organic compounds.

4. keeping in mind the above points translocation of non-exchangeable $^{3}H$ in organic compounds is similar to translocation of $^{14}C$ in organic compounds.

## REFERENCES


1. Aronoff, S., and I. C. Choi. 1963. Specific activity of photosynthetic sugars in soybean leaves equilibrated with tritiated water. Arch. Biochem. Biophys. 102:159-160.

2. Biddulph, O., and R. Corry. 1957. An analysis of translocation in the phloem of the bean plant using THO, $p^{32}$, and $C^{14}$. Plant Physiol. 32:608-619.

3. Bulley, N. R., C. D. Nelson, and E. B. Tregunna. 1969. Photosynthesis: Action spectra of leaves at normal and low oxygen. Plant Physiol. In press.

4. Choi, I. C., and S. Aronoff. 1966. Photosynthate transport using tritiated water. Plant Physiol. 41:1119-1129.

5. Dent, C. E. 1947. The amino-aciduria in Fanconi Syndrome. A study making extensive use of technique based on paper partition chromatography. Biochem. J. 41:422-427.

6. Forrester, M. L., G. Krotkov and C. D. Nelson. 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. Plant Physiol. 41:422-427.

7. Gage, R. S., and S. Aronoff. 1960. Translocation III. Experiments with carbon 14, chlorine 36, and hydrogen 3. Plant Physiol. 35:53-64.

8. Moses, V., and M. Calvin. 1959. Photosynthesis studies with tritiated water. Biochem. Biophys. Acta. 33:297-312.

9. Nelson, C. D., H. Clauss, D. C. Mortimer and P. R. Gorham. 1961. Selective translocation of products of photosynthesis in soybean. Plant Physiol. 36:581-588.

10. Nichiporovich, A. A., T. F. Andreyeva, N. P. Voskresenskaya, L. A. Nezgovorova, and Y. I. Novitzkiy. 1958. Various ways of transformation of carbon assimilated by plants in the process of photosynthesis. Proceedings of the First (UNESCO) International Conference. Paris 1957. Radioisotopes in Scientific Research. London. 4:411-431.

11. Plaut, Z., and L. Reinhold. 1967. The effect of water stress on the movement of $C^{14}$-sucrose and of tritiated water within the supply leaf of young bean plants. Aust. J. Biol. Sc. 20:297-

12. Racusen, D. W., and S. Aronoff. 1954. Metabolism of soybean leaves VI. Exploratory studies in protein metabolism. Arch. Biochem. Biophys. 51:68-78.

13. Sen, S. P., and A. C. Leopold. 1956. Influence of light and darkness upon carbon dioxide fixation. Plant Physiol. 31:323-329.

14. Shatenshtein, A. I. 1962. Isotopic exchange and replacement of hydrogen in organic compounds. Consultants Bureau Enterprises, Inc. New York.

15. Trip, P., and P. R. Gorham. 1968(a). Bidirectional translocation of sugar in sieve tubes of squash plants. Plant Physiol. 43:877-882.

16. Trip, P., and P. R. Gorham. 1968(b). Translocation of sugar and tritiated water in squash plants. Plant Physiol. 43:1845-1849.

17. Vernon, L. P., and S. Aronoff. 1950. Metabolism of soybean leaves II. Amino acids formed during short term photosynthesis. Arch. Biochem. 28:179-186.

18. Vernon, L. P., and S. Aronoff. 1952. Metabolism of soybean leaves IV. Translocation from soybean leaves. Arch. Biochem. Biophys. 36:383-398.

19. Went, F. W. 1953. The effect of temperature on plant growth. Ann. Rev. Plant Physiol. 4:347-362.

# Chapter 4

## Effect of $O_2$ Concentration on the Assimilation and Translocation of $^{3}H$- and $^{14}C$-Organic Compounds in Soybean

# INTRODUCTION

Modern theories of translocation usually include metabolic control acting at the source of the translocated compounds e.g. the leaf, at the sink to which the compounds are translocated e.g. the roots, in the conducting vessels e.g. the phloem, or some combination of these three (8,6,7,10,14). Metabolic control can be mediated by environmental conditions at the sink or along the translocation route (10) e.g.; temperature on stem and petiole (11,12) or light on the lower leaves and stem (5). However at the source leaf it is difficult to separate a direct effect on the translocation process from an indirect effect through the process of photosynthesis.

Knowing the patterns of assimilation of $^{3}H$ and $^{14}C$ are similar and that $^{3}H$ and $^{14}C$ are translocated in sucrose at the same rate (Chapt. 3), this information can be used to study the effect of various factors on translocation in the same plant. Since $O_2$ concentration is known to affect photosynthesis (1,4,9) it was decided to determine if $O_2$ concentration affected translocation and if it did, was the effect direct or through photosynthesis? In the same plant using a sequential feeding, one isotope can label the products of photosynthesis at 21% $O_2$ and the other isotope can label the products of photosynthesis at altered $O_2$ concentration. Translocation of these labelled organic compounds can then be followed.

# MATERIALS AND METHODS

Primary leaves of soybeans grown as described in Chapt. 3 were offered $3.43 \times 10^{-8}$ moles (50 mc) of $^{3}H$ as $^{3}H_2O$ vapour and $1.05 \times 10^{-8}$ moles (0.68 μc) of $^{14}CO_2$ in a sequential feeding in a closed circuit feeding apparatus (Fig. 4.1). The apparatus contained: a plexiglass leaf chamber, an infra red gas analyzer (Beckman series 215), an $O_2$ electrode (Beckman 777), a geiger tube (Anton-Lionel type 6222), connected to a ratemeter (Nuclear Chicago 8731) and a recorder (Riken Denshi Model S P-J 2), flasks for generating $^{14}CO_2$ and $^{3}H_2O$, a column for the removal of water vapour (Drierite) which could be removed from the system previous to generating $^{3}H_2O$, and a pump to circulate the gas stream within the closed circuit. During generation of $^{3}H_2O$ vapour and $^{14}CO_2$ the leaf chamber could be by-passed from the circuit and different gas mixtures (containing 2%, 21% or 99% $O_2$, with 0.03% $CO_2$ and remainder $N_2$) passed through the leaf chamber.

One of the primary leaves of each soybean was placed in the leaf chamber for a 30 minute adaptation period in light from quartz-iodine lamps (750 watts) filtered through 10 cm of water to yield 2,000 ft-c measured with a Weston light meter. During this period, air was circulated across the leaf and the rate of net photosynthesis was measured using the infra red gas analyzer.

Two experimental plans were followed (Fig. 4.2). In feeding sequence A, a primary leaf was fed $^{3}H_2O$ vapour for 1 minute in air at 21% $O_2$, 0.03% $CO_2$. The chamber was then flushed with air containing 2%, 21% or 99% $O_2$,

Figure 4.1 Apparatus for sequential feeding of $^{3}H_2O$ and $^{14}CO_2$ to a primary leaf of soybean.

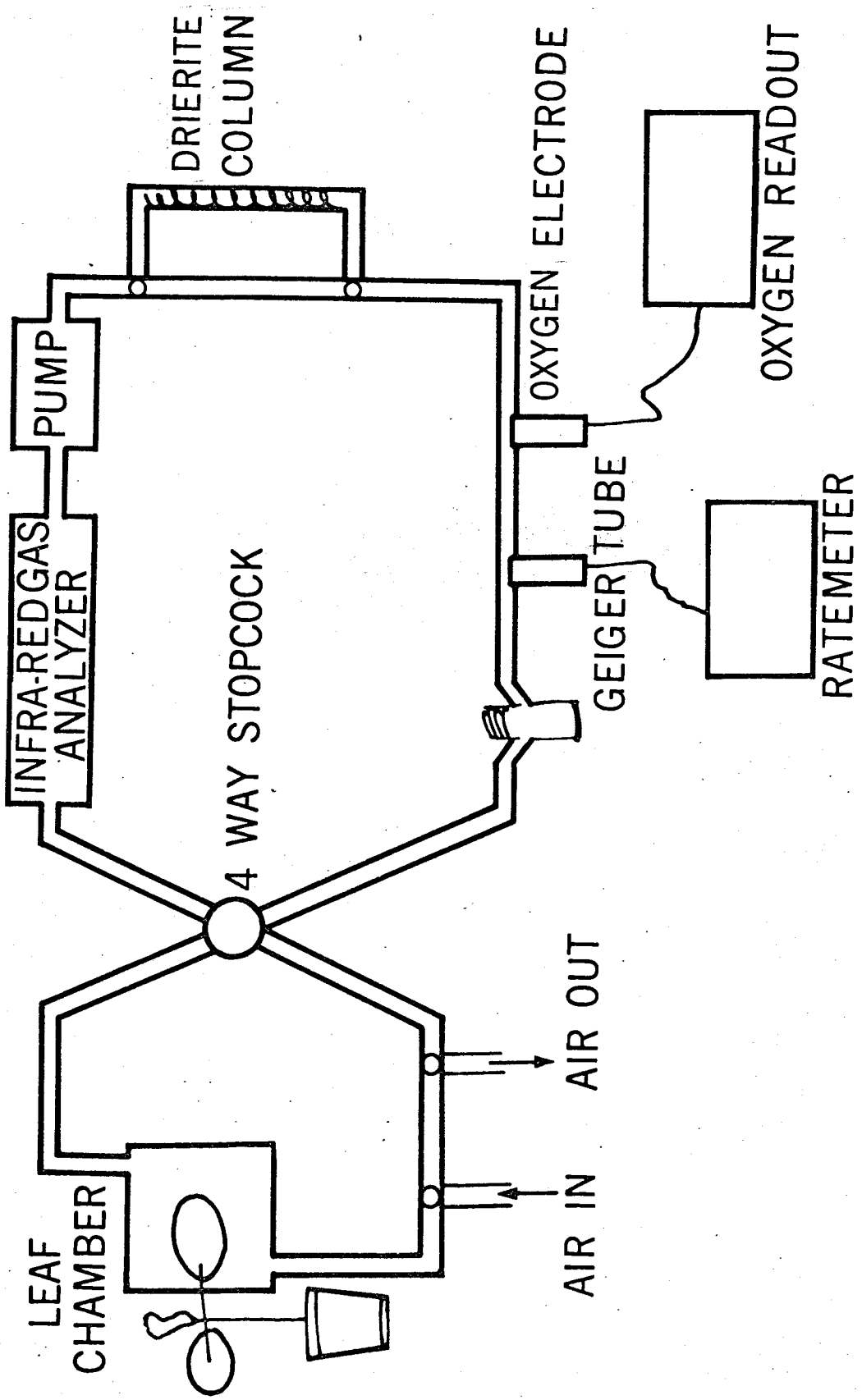
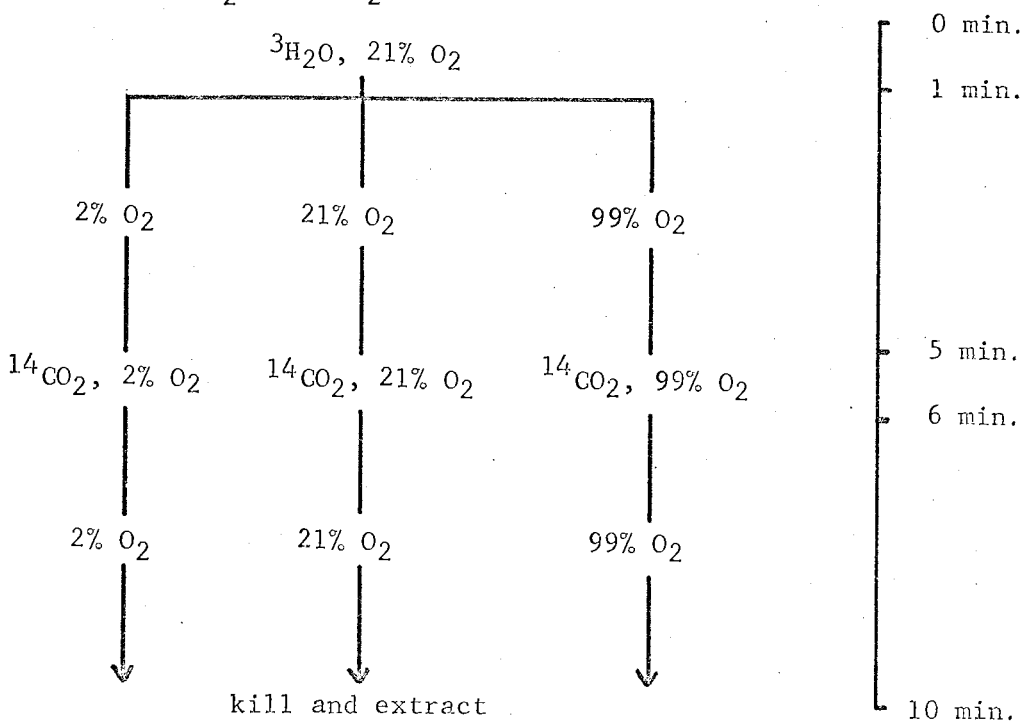
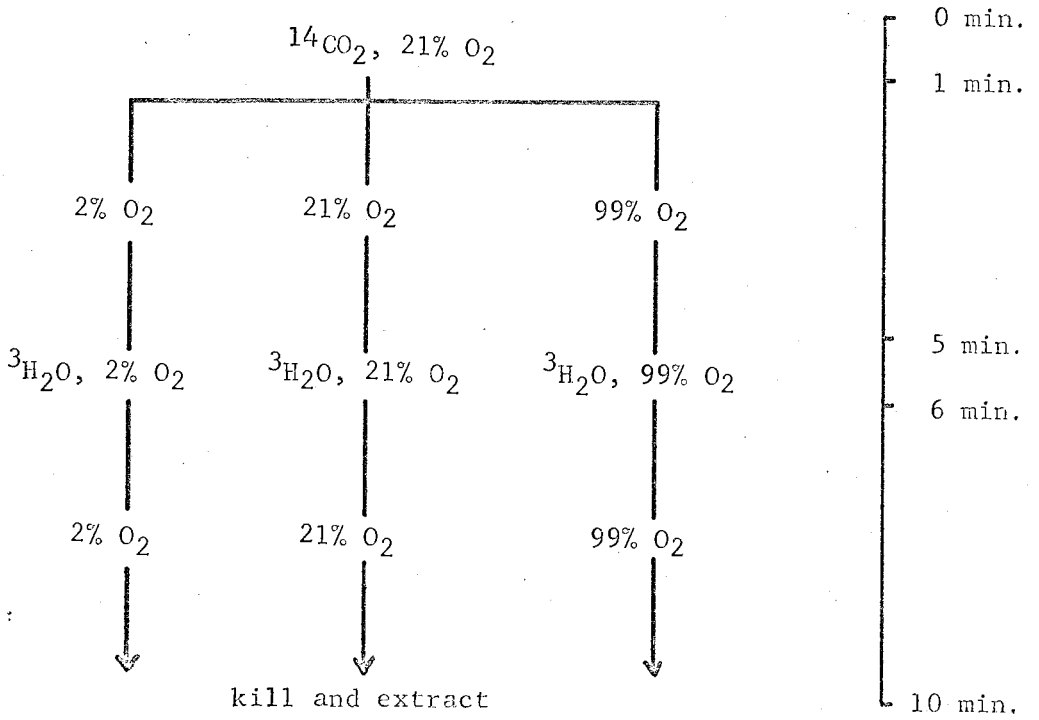

DRIERITE COLUMN
OXYGEN ELECTRODE
OXYGEN READOUT
PUMP
INFRA-RED GAS ANALYZER
4 WAY STOPCOCK
GEIGER TUBE
RATEMETER
AIR OUT
AIR IN
LEAF CHAMBER

Figure 4.2 Experimental sequence used to feed primary leaves of soybean $3.43 \times 10^{-8}$ moles (50 mc) of $^{3}H$ as $^{3}H_2O$ and $1.05 \times 10^{-8}$ (0.68 μc) $^{14}C$ as $^{14}CO_2$. The gas mixtures used during feeding contained 2%, 21% or 99% $O_2$, 0.03% $CO_2$ and the remainder $N_2$.

FEEDING SEQUENCE A $^{3}H_2O$ : $^{14}CO_2$




FEEDING SEQUENCE B $^{14}CO_2$ : $^{3}H_2O$



0.03% $CO_2$, and after 4 minutes $^{14}CO_2$ was added to the air stream and offered to the leaf for 1 minute. The $^{14}CO_2$ was then removed from the air stream by flushing with the gas mixture appropriate to the sequence and after 4 minutes the leaf was killed in boiling 80% ethanol : 20% water. Feeding sequence B was identical to feeding sequence A except the time of introduction of $^{3}H_2O$ and $^{14}CO_2$ was reversed.

In both feeding sequences, the $O_2$ concentration of the complete circuit was changed within 30 seconds. At all times during, between and after feedings the $CO_2$ concentration did not vary from 0.03% by more than ± 0.005%.

The leaf and other plant parts were extracted separately and washed repeatedly with cold 80% ethanol : 20% water until the amount of $^{3}H$ was constant (see Chapt. 3). Ethanol-soluble extracts of the fed leaf were separated into sugars, amino acids and organic acids by resin column chromatography--Rexyn 101 H and 201 OH. The amounts of $^{3}H$ and $^{14}C$ were determined using a liquid scintillation spectrometer and a dioxane-based scintillation solution.

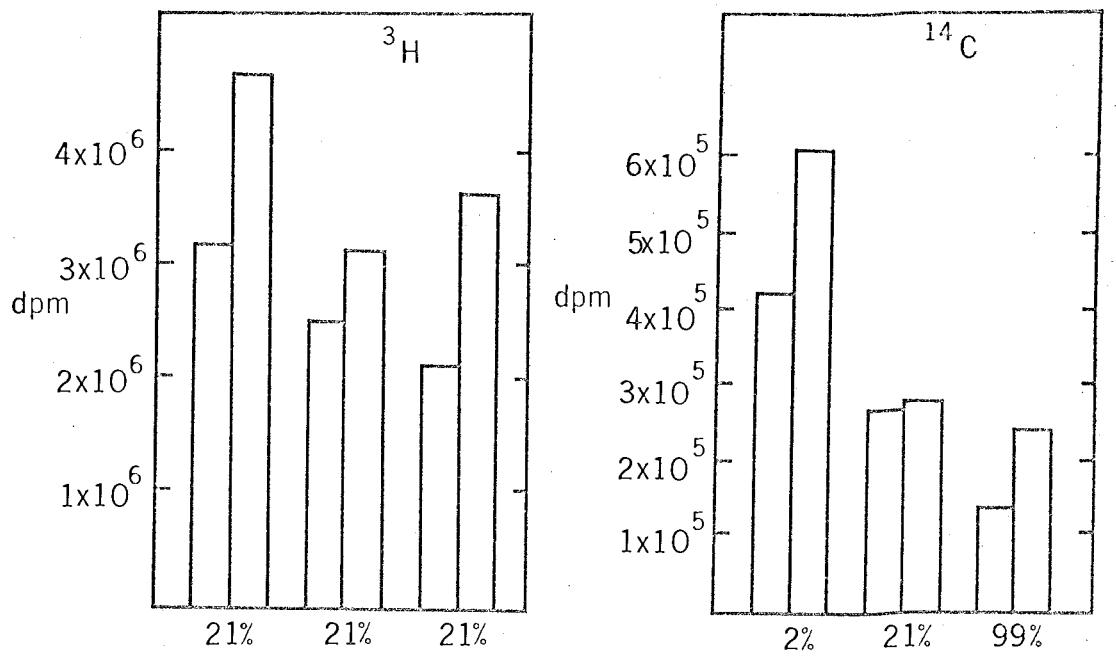
## RESULTS

Amounts of $^{3}H$ and $^{14}C$ assimilated into the organic compounds varied with the $O_2$ concentration during the feedings (Fig. 4.3). When $^{14}CO_2$ was fed at 21% $O_2$ (feeding sequence B) the amount of $^{14}C$ assimilated was found to be constant with increasing $O_2$ concentration. When $^{3}H_2O$ was fed at 21% $O_2$ (feeding sequence A), variation between plants was large enough that no significant trend in $^{3}H$ assimilation was apparent when the feeding was followed by 2%, 21% or 99% $O_2$. When $^{14}CO_2$ was fed at 2%, 21% or 99% $O_2$ (feeding sequence A) the amount of $^{14}C$ assimilated was found to decrease with increasing $O_2$ concentration. When $^{3}H_2O$ was fed at 2% and 99% $O_2$ (feeding sequence B) more than twice as much $^{3}H$ was assimilated as when $^{3}H$ was fed at 21% $O_2$.

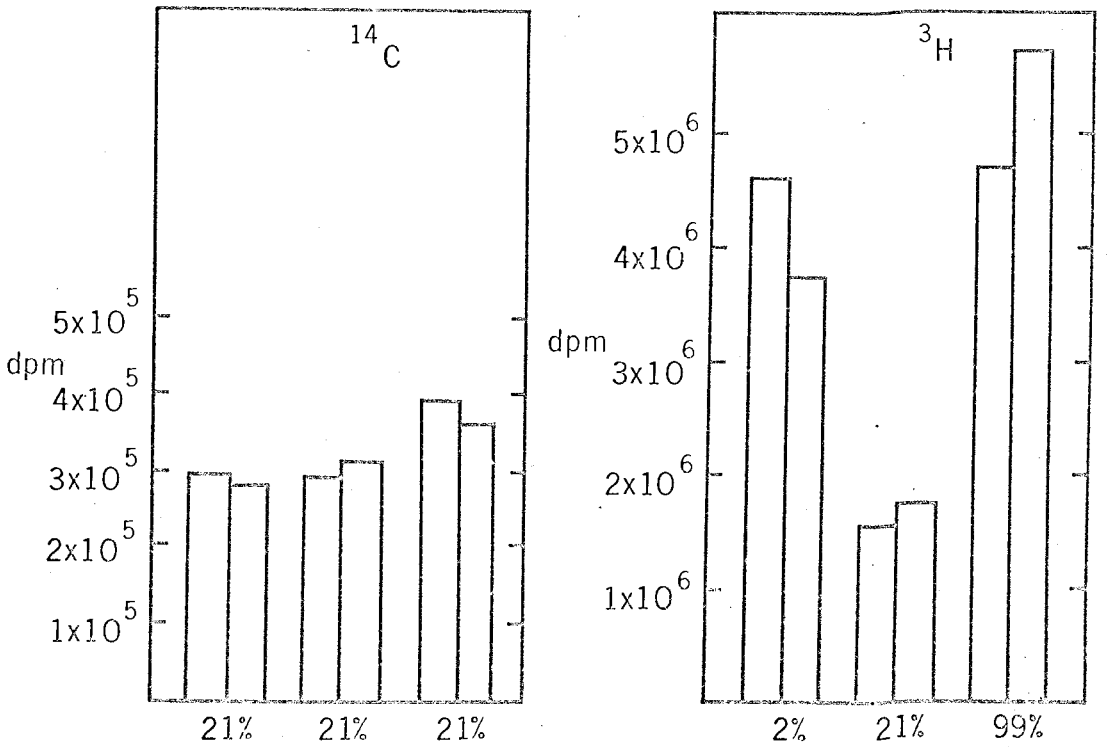
In feeding sequence A (Fig. 4.4) and sequence B (Fig. 4.5) the percentages of $^{3}H$ and $^{14}C$ distributed among the sugars, amino acids and organic acids differed with the $O_2$ concentrations. These results should be considered together for the most effective comparison. Both feeding sequences A and B present similar distribution with 21% $O_2$ no matter which isotope was offered first. However, there are differences between the two feeding sequences when treatment included 2% and 99% $O_2$. When the initial feeding was followed by 2% $O_2$ (Fig. 4.4A and 4.5A), both isotopes produced similar distributions, but when followed by 99% $O_2$, the proportion in sugars was markedly depressed with respect to $^{3}H$ (Fig. 4.4C). The organic and amino acids had proportions essentially equal to sugars (Fig. 4.4C).

Figure 4.3 Total non-exchangeable $^{3}H$ and $^{14}C$ assimilated into soybeans during feeding sequence A and B (Fig. 4.2). Each bar histogram represents one plant and each plant is represented twice; once for $^{3}H$ and again in the corresponding position in the $^{14}C$ figure.

FEEDING SEQUENCE, (a)




FEEDING SEQUENCE (b)




$[O_2]$ DURING FEEDING

Figure 4.4 Distribution of non-exchangeable $^{3}H$ and of $^{14}C$ among sugars, amino acids in the ethanol-soluble extracts of the primary leaves of soybean following a sequential feeding with $^{14}CO_2$ then $^{3}H_2O$, feeding sequence A (Fig. 4.2).

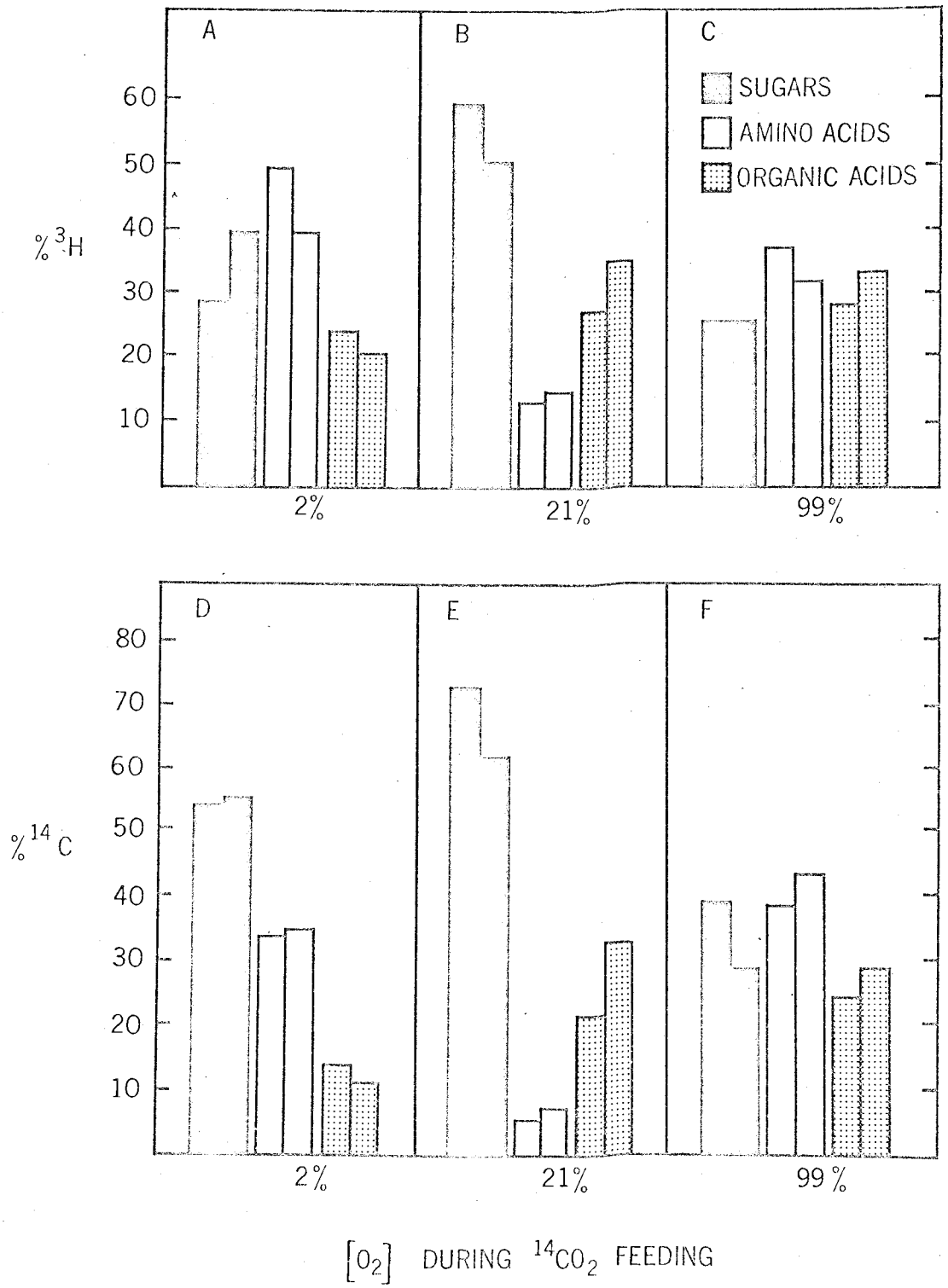
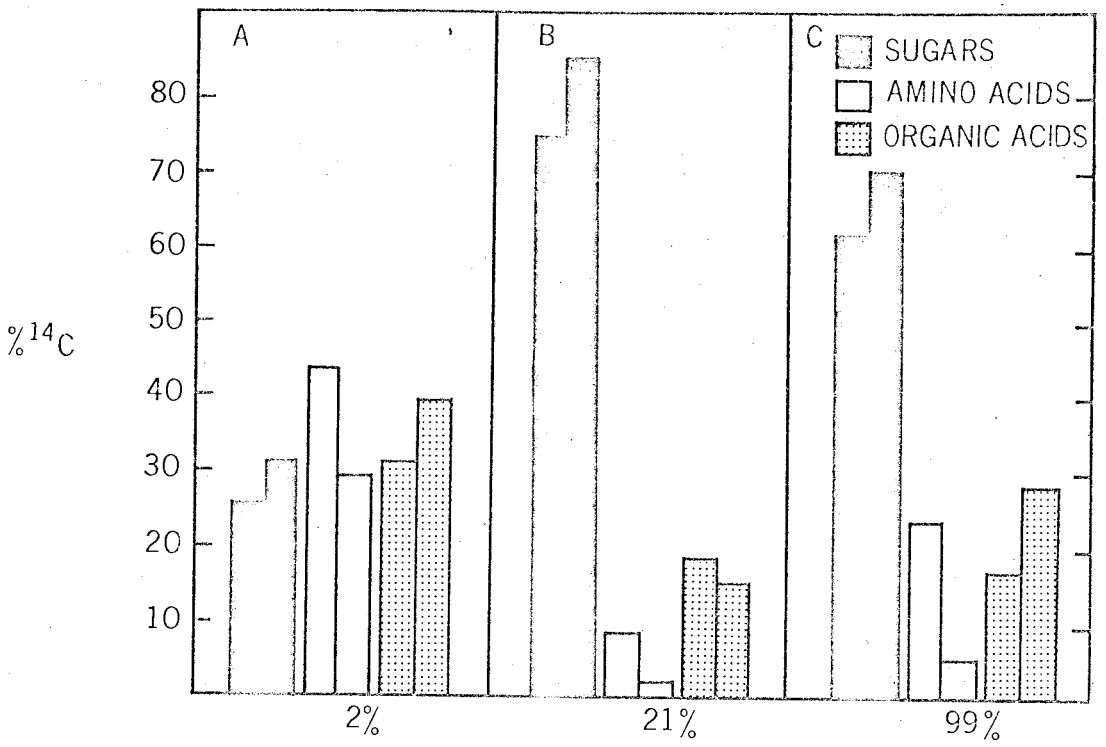
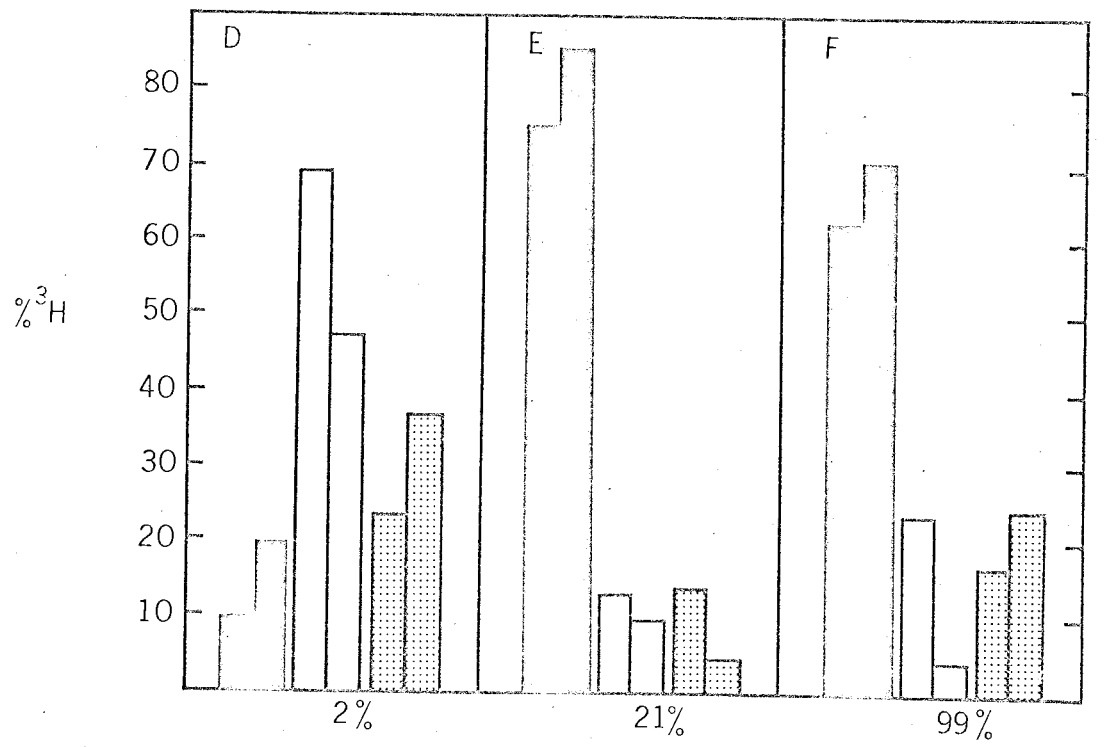

A
B
C
SUGARS
AMINO ACIDS
ORGANIC ACIDS
% 3H
60
50
40
30
20
10
2%
21%
99%
D
E
F
% 14 C
80
70
60
50
40
30
20
10
2%
21%
99 %
[O2] DURING 14CO2 FEEDING

Figure 4.5 Distribution of $^{14}C$ and non-exchangeable $^{3}H$ between sugars, amino acids and organic acids in the ethanol-soluble extract of the primary leaf of soybean following a sequential feeding with $^{14}CO_2$ then $^{3}H_2O$, feeding sequence B (Fig. 4.2).




[$O_2$] DURING $^3H_2O$ FEEDING

In the second part of the feeding sequences where the isotopes were presented during the altered environmental treatment, high amino acid labelling occurred with 2% and 99% $O_2$ for $^{14}C$ but only at 2% for $^{3}H$.

In other words, we note that the 2% $O_2$ treatment increases the proportion of $^{3}H$ and $^{14}C$ in the amino acids and organic acids in both feeding sequences A and B. The same pattern appeared with one pair of plants (Fig. 4.4) at the 99% $O_2$ but not with the second pair (Fig. 4.5) when the order of isotope administration was reversed.

Translocation of $^{3}H$- and $^{14}C$-organic compounds from the fed leaf was less than 10% of the $^{3}H$ and $^{14}C$ assimilated into the organic compounds for all $O_2$ concentration in both feeding sequences A and B (Table 4.1). At 2% $O_2$ in feeding sequence A lower percentages of $^{3}H$ and $^{14}C$ were translocated than at 21% and 99% $O_2$. At 2% $O_2$, in feeding sequence B, a lower percentage of the $^{3}H$ but a similar percentage of $^{14}C$ was translocated as the percentage of $^{3}H$ and $^{14}C$ translocated at 21% and 99% $O_2$. Thus translocation of $^{3}H$ was lower at 2% $O_2$. At 2% $O_2$ translocation of $^{14}C$ was also lower than at 21% or 99% $O_2$ if the $^{14}C$ was fed at 2% $O_2$ but was not lower when the $^{14}C$ was fed at 21% $O_2$ and followed by 2% $O_2$. At all $O_2$ concentrations, translocation of the first isotope fed in the sequence was always greater than the isotope fed 5 minutes later.

Table 4.1 Amount of $^{3}H$- and $^{14}C$-organic compounds translocated from the fed leaf as a percentage of total $^{3}H$ or $^{14}C$ in the plant. Each value is the mean of two plants.

| | Feeding sequence A[1]. $^{3}H_2O$ followed by $^{14}CO_2$ | | Feeding sequence B[1]. $^{14}CO_2$ followed by $^{3}H_2O$ | |
|---|---|---|---|---|
| $O_2$ concentration during second feeding | percent $^{3}H$ translocated after 10 min. | percent $^{14}C$ translocated after 5 min. | percent $^{14}C$ translocated after 10 min. | percent $^{3}H$ translocated after 5 min. |
| 2% | 0.8 | 0.6 | 2.4 | 1.1 |
| 21% | 5.8 | 3.5 | 2.8 | 2.3 |
| 99% | 7.1 | 3.2 | 2.9 | 2.8 |

1. see Fig. 4.2 for detailed description of feeding sequences.

# DISCUSSION

When a leaf that has been fed $^{14}CO_2$ is flushed with air, most of the unassimilated $^{14}CO_2$ is rapidly removed from the leaf (Chapt. 3, Table 3.1). If $^{14}CO_2$ is fed to the leaf at 21% $O_2$ and then is flushed with air enriched with $O_2$ it can be assumed that most of the assimilated $^{14}C$ was fixed at the original $O_2$ concentration. Thus the amount of $^{14}C$ assimilated at 21% was unaffected by the $O_2$ concentration following the feeding, but the amount of $^{14}C$ assimilated decreased inversely with $O_2$ concentration during feeding (Fig. 4.4) as reported previously for soybean (4).

Following a 1 minute feeding of $^{3}H_2O$, at least 5 minutes of flushing is required before all of the unassimilated $^{3}H_2O$ has been removed (Chapt. 3, Table 3.1). If $^{3}H_2O$ is fed to the leaf at 21% $O_2$ and then flushed with air at different $O_2$ concentrations, some of the $^{3}H$ will be assimilated at the new $O_2$ concentration. Assimilation of $^{3}H$ was lowest at 21% $O_2$ and increased at both 2% and 99% $O_2$ (Fig. 4.4). The increase at 99% $O_2$ is completely unexpected but may be accounted for in terms of postulates to follow. Assimilation at 21% $O_2$ followed by 2% and 99% $O_2$ was too variable to indicate if it followed the same trend.

At 21% $O_2$ in a simultaneous feeding the $^{3}H$ and $^{14}C$ initially found in the amino acids and organic acids was converted with time to sugars (Chapt. 3, Fig. 3.3). The results from the sequential feedings at 21% $O_2$ (Fig. 4.4 and 4.5) fit with a pathway in which amino acids and organic acids are converted to sugars (2). Conversion of amino acids and organic acids to sugars appears to be "blocked" at low $O_2$ concentration

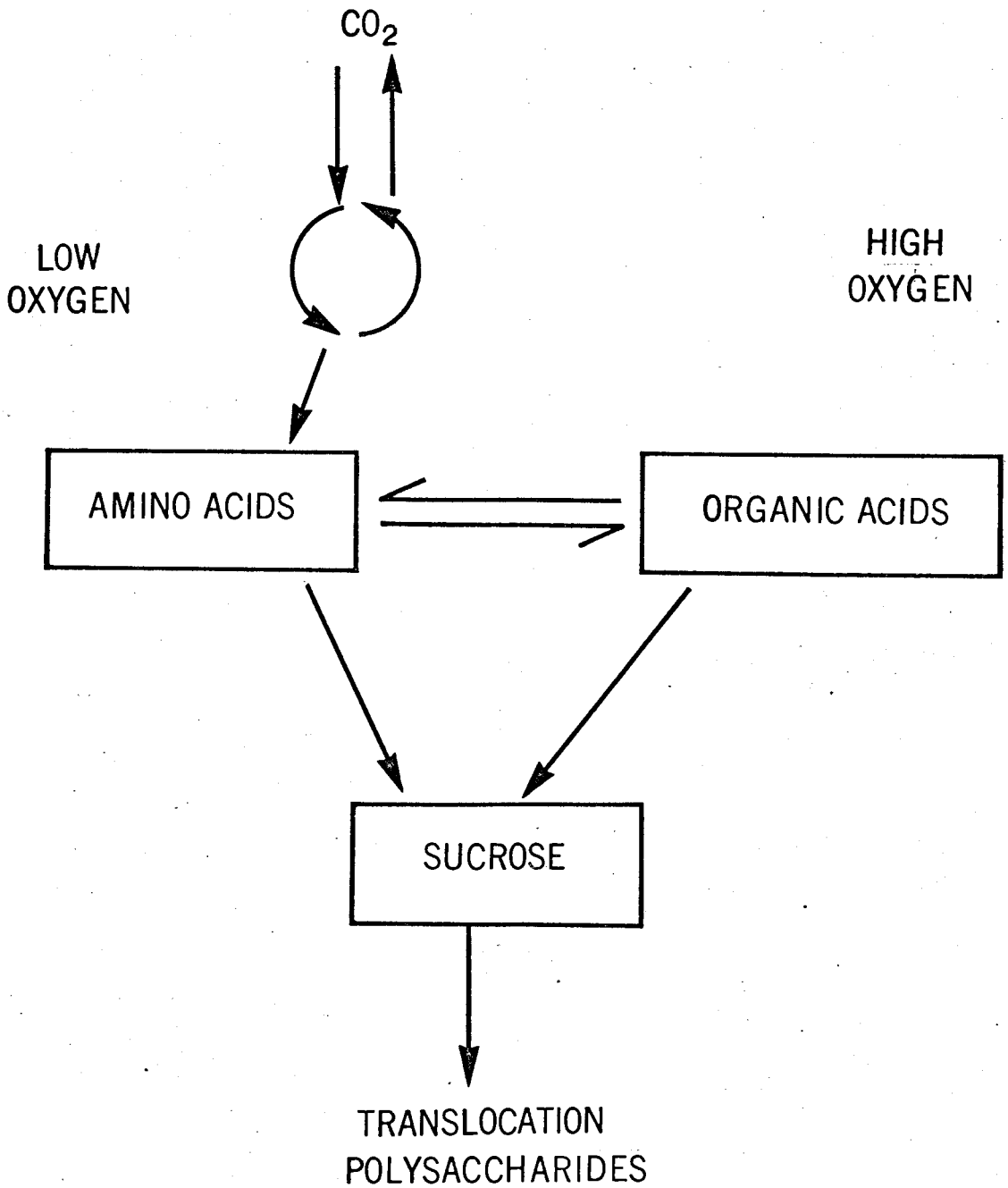
(Fig. 4.4 and 4.5) and also at high $O_2$ concentration in the plants in feeding sequence A (Fig. 4.4).

A schematic representation relating the effect of high and low $O_2$ concentration on carbon assimilation in photosynthesis is proposed in Fig. 4.6. At high $O_2$ concentration photorespiration is increased consequently increasing the loss of $CO_2$ and decreasing the net assimilation of carbon (4). Coombs and Whittingham (3) concluded that in Chlorella, high oxygen inhibited $CO_2$ assimilation by increasing the loss of carbon from the intermediates of a C-2 cycle rather than a specific inhibition of a particular enzyme. If $O_2$ increases the rate of oxidation of the C-2 fragments the equilibrium between metabolic reactions might be shifted so as to increase the amounts of organic acids and amino acids and decrease the amounts of sugar. Decreased amounts of sugar was found at high $O_2$ in Chlorella (3,13) as well as soybean (Fig. 4.4 and 4.5). If glutamic acid, containing a larger amount of $^3H$ than $^{14}C$ (Chapt. 3, Fig. 3.5), was favoured at high $O_2$ concentration this could account for increased amounts of $^3H$ assimilation at high $O_2$ in spite of the reduced carbon assimilation at high $O_2$ (Fig. 4.3).

At 2% $O_2$ concentration photorespiration ceases, consequently decreasing the loss of $CO_2$ and increasing the net assimilation of carbon (4). At low $O_2$ in Chlorella (13), as well as soybean (Fig. 4.4 and 4.5) amino acid production is increased. By favouring amino acids the equilibrium between metabolic reactions might be upset so as to decrease the amounts of sugar (Fig. 4.4 and 4.5).

In the relative short periods of time allowed for translocation, a

Figure 4.6 Schematic representation relating sugars, amino acids and organic acids at 2% and 99% $O_2$ to the metabolic pathway at 21% $O_2$.


$CO_2$
LOW
OXYGEN
HIGH
OXYGEN
AMINO ACIDS
ORGANIC ACIDS
SUCROSE
TRANSLOCATION
POLYSACCHARIDES

slower translocation rate was noticed with only 2% $O_2$. When $^{14}C$ was offered at 21% $O_2$ and followed by 2% $O_2$ translocation was not affected. When $^{3}H$ was offered at 21% $O_2$ and followed by 2% $O_2$, translocation was slower. However this might be expected since most of the $^{3}H$ was assimilated at 2% $O_2$ (Chapt. 3, Table 3.1). Thus low $O_2$ concentrations appear to alter translocation by altering assimilation patterns. Experiments for longer periods of time are needed to determine whether translocation is affected with 99% $O_2$, since it seems inconsistent that with both 99% $O_2$ and 2% $O_2$, assimilation patterns are altered so as to decrease the amount of sugars "available" for translocation but the rate of translocation is slowed with only 2% $O_2$.

So far in this discussion photosynthetic assimilation and translocation has been considered separately. By the use of more plants the same information can be obtained from conventional single isotope experiments. It was hoped that the isotope fed at 21% $O_2$ would act as a control for the experiment and the effect of $O_2$ on assimilation and translocation could be separated in the same plant. However decreasing or increasing the $O_2$ concentration affected the subsequent metabolism of the organic compounds making the first isotope fed a poor control for the experiment. The experimental design can be improved to make better use of the two isotopes by increasing the time between feedings and allowing the first isotope to complete the conversion from amino acids and organic acids to sugars before changing the $O_2$ concentration. In this way the first isotope becomes a true control for the experiment which then can be compared to the second isotope at reduced $O_2$ concentration.

In summary, at 2% $O_2$ the distribution of $^3H$ and $^{14}C$ among the organic compounds changes from the distribution at 21% $O_2$. At the same time there is a decrease in translocation of $^3H$- and $^{14}C$-organic compounds. At 99% $O_2$ the distribution of $^3H$ and $^{14}C$ among the organic compounds changes from the distribution at 21% $O_2$ and yet there is no change in translocation of $^3H$ and $^{14}C$-organic compounds. Thus a fairly complex relationship exists between amount of compounds available in the fed leaf for translocation and the amounts of compounds actually translocated. To completely understand this relationship further knowledge is needed concerning the effects of $O_2$ on the percentage of $^3H$ or $^{14}C$ distributed in the organic compounds at shorter intervals of time as well as determining if there is a long term inhibition of translocation at 2% $O_2$.

## REFERENCES


1. Bjorkman, O. 1966. The effect of oxygen concentration on photosynthesis in higher plants. Physiol. Plantarum. 19:618-633.

2. Burma, D. P., and D. C. Mortimer. 1957. The fate of assimilated $C^{14}O_2$ studied by displacement with $C^{12}O_2$. Can. J. Biochem. and Physiol. 35:835-843.

3. Coombs, J., and C. P. Whittingham. 1966. The effect of high partial pressures of oxygen on photosynthesis in Chlorella - I. The effect on end products of photosynthesis. Phytochem. 5:643-651.

4. Forrester, M. L., G. Krotkov and C. D. Nelson. 1966. Effect of oxygen on photosynthesis, photorespiration and respiration in detached leaves. I. Soybean. Plant Physiol. 41:422-427.

5. Habeshaw, D. 1969. The effect of light on translocation from sugar beet leaves. J. of Exp. Bot. 20:64-71.

6. Kursanov, A. L. 1963. Metabolism and the transport of organic substances in the phloem. In: Advances in Botanical Research Vol. I. Ed., R. D. Preston. Academic Press. N. Y. pp. 209-278.

7. Swanson, C. A. 1959. Translocation of organic solutes. In: Plant Physiology: A Treatise, Vol. II. Ed., F. C. Steward. pp. 481-551.

8. Thaine, R. 1964. Long distance transport in plants. World Rev. of Pest Control. 3:175-187.

9. Tregunna, E. B., G. Krotkov and C. D. Nelson. 1966. Effect of oxygen on rate of photorespiration in detached tobacco leaves. Physiol. Plantarum. 19:723-733.

10. Wardlaw, I. F. 1968. The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34:79-105.

11. Webb, J. A. 1967. Translocation of sugars in Cucurbita melopepo IV. Effects on temperature change. Plant. Physiol. 42:881-885.

12. Webb, J. A., and P. R. Gorham. 1965. The effect of node temperature on assimilation and translocation of $^{14}C$ in squash. Can. J. Bot. 43:1009-1020.

13. Zak, E. G. 1965. Influence of molecular oxygen on the formation of amino acids during photosynthesis in Chlorella under various conditions of illumination. Soviet Plant Physiol. 12:225-229.

14. Zimmermann, M. H. 1960. Transport in phloem. Ann. Rev. Plant Physiol. 11:167-190.

# SUMMATION OF THE THESIS

The bimodal pattern of translocation in grand fir with peak rates in spring and autumn makes the timing of application of systemic insecticides very important. Experiments carried out when translocation was low might give the impression the systemic insecticide was ineffective. The same insecticide might be more effective at another time of year. Translocation of $^{32}$P-schradan was found to be in the phloem of grand fir (1) but, all systemic insecticides may not be translocated in the same way as photosynthetically assimilated organic compounds. However, the mode of translocation of an insecticide could be determined by labelling it with a radioactive isotope and tracing its movement with a geiger tube. For insecticides translocated with a seasonal pattern, the geiger-tube technique could easily be adapted to determine peak rates of translocation under field conditions.

The large amounts of $^{14}$C-shikimic and $^{14}$C-quinic acids found in the spring tissue may indicate that cell metabolism is directed towards lignin formation and building cell walls. It would be interesting to pursue this further by determining if increased translocation in the spring is due to "leaky" cells and if this translocation could be induced at other periods of the year without too much damage to the tissue.

The technique of using non-exchangeable $^{3}$H and $^{14}$C to label the organic compounds in translocation experiments appears useful in establishing the effect of environmental factors on the control of translocation. It may be particularly important in plants which show considerable amount of individual variation in rates of translocation such as trees grown under field conditions.

Glutamic acid and glycine-serine were the exceptions to the $^{3}H$- and $^{14}C$-labelling patterns in the photosynthetically assimilated organic compounds. The greater percentage of $^{3}H$ in glutamic acid, than expected on the basis of the percentage of $^{14}C$, may be a clue to the assimilation of $^{3}H$ into the organic compounds and could be worth further experimentation. The greater percentage of $^{14}C$ in glycine-serine, than expected on the basis of the percentage of $^{3}H$, could indicate a compartmentalization of glycine-serine in an area of the cell which is "close" to the first products of photosynthesis but is "remote" from water entering the cytoplasm. These ideas could also be worth further development.

Seasonal variations in rates of translocation of organic compounds is important from both practical and theoretical aspects. The geiger-tube technique and the dual-isotope labelling technique may prove useful to study translocation at this level.

## REFERENCE

1. Rediske, J. H., and N. E. Johnson. 1965. The absorption and translocation of the systemic insecticide schradan in sitka spruce and grand fir. Weyerhaeuser Forestry Paper No. 5. Centralia, Wash.

# APPENDIX 1

## Efficiency of the Anton-Lionel Miniature Counting Tube Type 6222

Previous to using the Anton-Lionel geiger tube for experiments with the grand firs and again after the series of experiments were completed, the counting efficiency of the tube was checked against aliquots of $^{14}C$-aspartic acid (0.1 mc per 1.0 ml) dried on a glass cover slip. To obtain a concept of the amount of shielding by the tree, spots 1/4 inch in diameter cut from paper chromatograms and containing $^{14}C$-organic compounds were counted with the Anton-Lionel geiger tube. The diameter of the end window of the geiger tube is 1/4 inch.

Although the operating voltage had increased from 700 to 750 volts the efficiency of the geiger tube did not change from beginning of the experiments to the end (Table A, Appendix 1). The mean efficiency of 19.5% is probably not significantly less than the 20.0% efficiency quoted by the company.

The chromatography paper shielded a considerable amount of the radioactivity from the geiger tube. However efficiency of counting chromatogram spots (Table A, Appendix 1) is still higher than efficiency of counting $^{14}C$ in the branch (Table 1.3, Chapt. 1) since very little of the cylindrical branch is against the flat surface of the geiger tube.

Table A. The efficiency of the Anton-Lionel geiger tube to measure $^{14}$C-aspartic acid dried to a glass cover slip and $^{14}$C-organic compounds on chromatography paper.

| | | efficiency | operating voltage |
|---|---|---|---|
| $^{14}$C-aspartic acid dried to a glass cover slip. | (beginning of experiments[1.] | 19.6 ± 3% | 700 |
| | (end of experiments[1.] | 19.5 ± 4% | 750 |
| $^{14}$C-organic compounds on chromatography paper | [2.] | 1.38 ± 7% | 750 |

1. mean of three values ± coefficient of variation.

2. mean of eight values ± coefficient of variation.

# APPENDIX 2

## Distribution of Ethanol-Soluble $^{14}C$-Organic Compounds in Grand Firs

Grand firs (Abies grandis Dougl.) as described in Chapt. 1 were fed 135.1 μc of $^{14}CO_2$ for 2 and 2 1/2 hours during the spring to autumn of 1967 using the apparatus described in Chapt. 1. The length of time the trees were left in air between beginning of feeding and extraction with 80% ethanol : 20% water varied between 2 hours and 48 hours. These times are recorded in Table B, Appendix 2. Only the ethanol-soluble extract was assayed for $^{14}C$.

In all feedings during the season over 90% of the ethanol-soluble $^{14}C$ remained in the fed branch (Table B, Appendix 2). Less than 2% of the ethanol-soluble $^{14}C$ was found in the roots after 24 and 48 hours in May feedings. In white pine after 8 hours in May the percentage of ethanol-soluble $^{14}C$-compounds translocated to the roots varied between 5% and 26% of total ethanol-soluble $^{14}C$ found in the tree depending on whether the trees were grown at high light and high moisture (1,2). Greater translocation to the roots found in white pine as compared to balsam fir could be due to:

1. Increased translocation to the roots, the white pine were kept at $CO_2$ compensation point after feeding with $^{14}CO_2$, while the balsam firs were kept in air.
2. All the shoot above the roots of the white pine was offered $^{14}CO_2$ while only a 1 cm section of a balsam fir branch from the top whorl

of branches was offered $^{14}CO_2$.

3. The white pine and balsam fir belong to different genera and had different conditions of growth previous to the experiments.

## REFERENCES

1. Lister, G. R., V. Slankis, G. Krotkov and C. D. Nelson. 1967. Physiology of Pinus strobus L. seedlings grown under high or low soil moisture conditions. Annals of Bot. 31:121-132.

2. Shiroya, T., G. R. Lister, V. Slankis, G. Krotkov and C. D. Nelson. 1966. Seasonal changes in respiration, photosynthesis and translocation of the C labelled products of photosynthesis in young Pinus strobus L. plants. Annals of Bot. 30:81-91.

Table B. Distribution of ethanol-soluble $^{14}C$ in various sections of grand firs following a feeding of $^{14}CO_2$ to a 1 cm section of a branch in the upper whorl of branches. Each value is the percentage of total ethanol-soluble in that section of tree.

| Feeding date | | May 28 | May 28 | June 26 | July 6 | Aug 15 | Sept 13 |
|---|---|---|---|---|---|---|---|
| Hours from beginning of feeding to extraction | | 24 | 48 | 6 | 2 | 12 | 24 |
| Hours offered $^{14}CO_2$ | | 2 1/2 | 2 1/2 | 2 | 2 | 2 | 2 |
| fed branch | (above fed region | - | - | 0.2 | 0.1 | 4.2 | 9.2 |
| | ( fed region | 69.3 | 74.9 | 99.7 | 98.4 | 68.6 | 62.8 |
| | (below fed region | 25.0 | 20.7 | 0.2 | 0.2 | 24.4 | 18.8 |
| upper whorl of branches | (growth during current season | 3.3 | 2.9 | 0.03 | 0.4 | 1.9 | 1.6 |
| | (growth during previous season | 0.7 | 1.0 | 0.02 | 0.2 | 0.07 | 1.8 |
| lower whorl of branches | (growth during current season | 0.2 | 0.2 | 0.06 | 0.7 | 0.1 | 1.6 |
| | (growth during previous season | 0.1 | 0.3 | 0.0 | 0.0 | 0.2 | 2.4 |
| roots | | 1.4 | 0.2 | 0.0 | 0.0 | 0.5 | 1.9 |

# APPENDIX 3

## Elution of $^{3}H$- and $^{14}C$-Organic Compounds from Chromatogram Spots

Plant extracts were separated into individual organic compounds using paper chromatography. To assay for the amount of $^{3}H$ and $^{14}C$, each organic compound was eluted from its chromatogram spot and the eluate was counted in the liquid scintillation spectrometer using a dioxane scintillation solution. The time taken and the number of drops necessary to elute 99% of the $^{3}H$- and $^{14}C$-organic compounds from chromatogram spots containing sucrose, glucose, fructose, aspartic acid-malic acid, glutamic acid, glycine-serine and asparagine was determined previous to working with the experimental extracts.

Aliquots of an extract from a primary leaf of 19-day-old soybean which had been offered $^{3}H_2O$ and $^{14}CO_2$ for 30 minutes were separated into individual organic compounds using two dimensional paper chromatography. The position of the radioactive spots on the paper chromatogram were determined from radioautograms of the chromatogram and by spraying replicate chromatograms with ninhydrin and benzidine sprays. Chromatogram spots were cut in ovals with one end tapered to a point from which the eluate could drip. Wicks made from strips of chromatogram paper were stapled to the spots and the top of the wick was dipped into a 10 ml beaker containing distilled water. Successive drops were collected in individual vials containing scintillation solution. The 10 ml beaker was supported on a block of wood 4 inches high and the block, vial and 10 ml beaker were

enclosed under a inverted 1000 ml beaker. After seven drops had been eluted from the chromatogram spot, the spot was dropped into a vial containing scintillation solution and counted in the liquid scintillation spectrometer. The amount of $^{3}H$ in each drop was expressed as percent of total $^{3}H$ eluted from the chromatogram spot plus the residual $^{3}H$ on the chromatogram spot after the seven drops were eluted. A similar percentage was calculated for the amount of $^{14}C$ in each drop.

Over 60% of the total $^{3}H$ and $^{14}C$ was eluted in the first drop from the chromatogram spot for all compounds tested (Table C, Appendix 3). A rapid drop in the amount of $^{3}H$ and $^{14}C$ eluted after the first 3 drops was characteristic of all compounds and within 6 drops 99% of the $^{3}H$ and $^{14}C$ was eluted. The time taken for each drop to leave the chromatogram was variable. The first drop took from 15 to 30 minutes to leave the chromatogram spot and subsequent drops took less time. All 7 drops took from 50 to 100 minutes to fall.

On the basis of these test drops, in the experimental procedure 7 drops were eluted from each chromatogram spot into the same scintillation vial. This was about 99% of the total $^{3}H$ and $^{14}C$ present in each spot.

Table C. Percentage of $^{3}H$ and $^{14}C$ eluted from the chromatogram spots with each drop of water. Each value is expressed as a percentage of the total $^{3}H$ or $^{14}C$ eluted from the chromatogram spot plus the residue $^{3}H$ or $^{14}C$ left on the chromatogram spot after 7 drops were eluted.

| | sucrose | glucose | fructose | aspartic-malic acid | glutamic acid | glycine-serine | asparagine |
|---|---|---|---|---|---|---|---|
| Total $^{3}H$ dpm | 5,460,000 | 1,180,000 | 651,000 | 912,000 | 2,080,000 | 397,000 | 912,000 |
| drop no. 1 | 84.0% | 79.0% | 83.5% | 77.5% | 58.5% | 79.0% | 67.5% |
| 2 | 12.5 | 17.0 | 13.0 | 15.5 | 31.0 | 13.0 | 19.5 |
| 3 | 2.5 | 3.0 | 1.0 | 3.5 | 4.5 | 3.0 | 2.5 |
| 4 | 0.5 | 0.5 | 0.5 | 1.0 | 2.5 | 1.5 | 6.0 |
| 5 | 0.1 | 0.0 | 0.5 | 1.0 | 2.0 | 1.5 | 2.5 |
| 6 | 0.1 | 0.0 | 0.5 | 0.5 | 1.0 | 1.5 | 1.5 |
| 7 | 0.0 | 0.0 | 0.5 | 0.5 | 0.0 | 0.5 | 0.5 |
| residue | 0.3 | 0.5 | 0.5 | 0.5 | 0.5 | 0.0 | 0.0 |
| Total $^{14}C$ dpm | 327,000 | 12,600 | 5,400 | 10,800 | 16,200 | 3,610 | 14,300 |
| drop no. 1 | 83.0% | 79.0% | 80.0% | 79.0% | 62.5% | 78.5% | 65.0% |
| 2 | 11.5 | 16.5 | 16.5 | 13.0 | 31.0 | 13.0 | 18.5 |
| 3 | 4.5 | 3.0 | 1.0 | 4.5 | 3.5 | 3.0 | 7.5 |
| 4 | 0.5 | 0.5 | 0.5 | 2.0 | 1.0 | 1.5 | 5.0 |
| 5 | 0.0 | 0.0 | 0.5 | 0.5 | 1.0 | 1.5 | 2.5 |
| 6 | 0.0 | 0.0 | 0.5 | 0.5 | 0.5 | 1.5 | 0.5 |
| 7 | 0.0 | 0.0 | 0.5 | 0.0 | 0.0 | 0.5 | 0.5 |
| residue | 0.5 | 1.0 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |

# CURRICULUM VITAE

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. Honours (Biology-Chemistry) 1964
School of Graduate Studies 1964-1965

Simon Fraser University
Graduate Studies 1965-1966
M.Sc. 1966
Graduate Studies 1966-1969

Experience:

Laboratory Demonstrator
Department of Biology
Queen's University, Kingston 1964-1965

Tutorial Instructor
Department of Biological Sciences
Simon Fraser University 1965-1969

Awards: Government of Ontario Fellowship 1964-1965