DEVELOPMENTAL CHANGES IN ASSIMILATION AND TRANSLOCATION OF PHOTOSYNTHATE IN BLACK WALNUT (JUGLANS NIGRA L.) AND HONEYLOCUST (GLEDITSIA TRIACANTHOS L.) SEEDLINGS

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This is to certify that the

thesis entitled

Developmental changes in assimilation and translocation of Photosynthate in black walnut (Juglans Nigra L.) and honeylocust (Gleditsia Triacanthos L.) seedlings

presented by

Stanley Barton Carpenter

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of the requirements for

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ABSTRACT

DEVELOPMENTAL CHANGES IN ASSIMILATION AND TRANSLOCATION OF PHOTOSYNTHATE IN BLACK WALNUT (JUGLANS NIGRA L.) AND HONEYLOCUST (GLEDITSIA TRIACANTHOS L.) SEEDLINGS

By

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Growth patterns, rates of photosynthesis and respiration, and the incorporation of photoassimilated $^{14}CO_2$ into major fractions of the leaves, stem, and roots were studied in black walnut and honeylocust seedlings during the first growing season. Black walnut is typical of those deciduous trees with the preformed shoot growth habit whereas honeylocust has the sympodial growth habit. Other differences between the two species include seed size, leaf dimorphism, and nastic leaf movement.

Analyses of cumulative height growth, leaf area accretion, and dry matter production showed height growth in black walnut was of short duration and was completed early in the growing season. In contrast, honeylocust seedlings grew continuously in height through the summer to September 1. Similar trends were observed in leaf area accretion and dry matter production. However, root growth in both species occurred to September 21.

Rates of photosynthesis and respiration were determined in the laboratory with an infrared-gas analyzer in a closed system. Honeylocust seedlings exhibited a remarkable superiority over black walnut in rates of net photosynthesis computed on a leaf area basis. Net photosynthesis ranged as high as 14 mg CO_2 dm^{-2} hr^{-1} for black walnut and as high as 20 mg CO_2 dm^{-2} hr^{-1} for honeylocust. However, when net or total photosynthesis was calculated on a whole seedling basis, black walnut because of its larger leaf area showed much higher rates of CO_2 uptake. Net photosynthesis increased gradually in honeylocust seedlings and reached a peak incorporation of 15.5 mg CO_2 dm^{-2} hr^{-1} on August 10 and remained at a high level through September 21 as leaf abscission began. Net photosynthesis in black walnut reached a peak level of incorporation of 7.8 mg CO_2 dm^{-2} hr^{-1} on July 27 and then declined sharply to a low level of 1.3 mg CO_2 dm^{-2} hr^{-1} on September 7. There was considerable variation in rates of net photosynthesis, dark respiration, and photorespiration among individual trees of both species. Rates of CO_2 evolution for dark respiration were as high as 10 mg CO_2 dm^{-2} hr^{-1} for black walnut and 13 mg CO_2 dm^{-2} hr^{-1} for honeylocust. Photorespiration rates ranged as high as 9 mg CO_2 dm^{-2} hr^{-1} in black walnut and 22 mg CO_2 dm^{-2} hr^{-1} for honeylocust.

Distinct differences were observed in the pattern of ^{14}C incorporation into amino acid, organic acid, sugar, non-water soluble, and ethanol-insoluble fractions extracted

from the leaves, stem, and roots. In black walnut seedlings there was a gradual shifting of metabolic activity from the leaves to the stem and finally to the roots as the growing season progressed. In contrast the pattern of metabolism and translocation in honeylocust seedlings remained static. The leaves of walnut seedlings labeled early in the growth cycle showed reduced levels of radioactivity as leaf abscission began in the fall indicating redistribution of metabolites when leaf abscission occurs. In contrast high levels of ^{14}C remained in honeylocust leaves on September 21 as leaf abscission began. The study of the incorporation of $^{14}CO_2$ into specific sugars of black walnut showed distinct differences when a period of maximum photosynthetic activity was compared with a period of low photosynthetic activity. Sucrose accounted for 66 per cent of the total radioactivity recovered on June 15. Smaller amounts of radioactivity were found in glucose, fructose, raffinose, and stachyose. On September 7 the sucrose fraction contained only 44 per cent of the total ^{14}C recovered from walnut seedlings. Increased amounts of radioactivity were found in glucose, fructose, raffinose, and stachyose.

DEVELOPMENTAL CHANGES IN ASSIMILATION AND TRANSLOCATION OF PHOTOSYNTHATE IN BLACK WALNUT (*JUGLANS NIGRA* L.) AND HONEYLOCUST (*GLEDITSIA TRIACANTHOS* L.) SEEDLINGS

By

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INTRODUCTION

Deciduous hardwood species dominate the eastern half of the United States yet surprisingly little is known of their growth and metabolism. Important physiological processes in most valuable hardwood species are virtually unstudied. More research has probably gone into the elimination of hardwoods and their subsequent replacement with conifers than towards increasing our knowledge of their basic physiology.

Despite their large size, forest trees characteristically exhibit low rates of photosynthesis when compared with crop plants (Jarvis and Jarvis, 1964; Larcher, 1969). Comparative photosynthetic rates of deciduous and coniferous trees have seldom been measured under similar conditions. Deciduous trees would appear to be at a distinct disadvantage in terms of growth because of their deciduous habit. Photosynthesis in deciduous hardwoods is of course limited to the time that the leaves are present and perhaps only a portion of this period. Maximum rates of photosynthesis are achieved only after the leaves reach full size (Shiroya, et al., 1961). Leaf abscission is

preceded by a period of gradually increasing senescence of the leaves during which photosynthesis has been shown to be greatly reduced (Rhoads and Wedding, 1953). On the other hand photosynthesis in coniferous species occurs throughout the year during favorable conditions. Photosynthesis has been observed by Freeland (1945) in Pinus sylvestris L., Picea mariana (Mill.) B.S.P., and Pinus nigra Arnold during the winter when temperatures ranged as low as -6°C. Others have also detected photosynthesis and respiration at temperatures below freezing (Brown, 1970; Parker, 1953).

Available data indicate that deciduous hardwoods are not disadvantaged by the limitations of a leafless period. They may grow and photosynthesize at greater rates than conifers allowing for greater dry matter accumulation over the period of a year. Verduin (1953) projected photosynthetic rates of Picea pungens Engelm. to be only one-seventh the rates reported for apple. Jarvis and Jarvis (1964) observed that maximum rates of net photosynthesis for temperate zone conifers were in the range of 5 to 10 mg CO_2 dm^{-2} hr^{-1} compared to rates of 10 to 20 mg CO_2 dm^{-2} hr^{-1} for broad-leaved trees and shrubs. Kramer (1953) reported (work of Polster in Germany) that the average rate of photosynthesis of evergreen conifers for an entire season is considerably lower than that of broad-leaved species, and the rate of deciduous larch is intermediate.

Kramer and Decker (1944) in comparative studies of hardwoods and pines attributed the lower rates of photosynthesis of the pines to mutual shading of the needles. Krueger and Ferrell (1965) found maximum rates of net photosynthesis in young Douglas-fir seedlings to be as high as 18 mg CO_2 dm^{-2} hr^{-1}. Brix (1967) also reported similar high rates for this species. Many studies have shown that broadleaf species attain maximum photosynthesis at lower light intensities than conifers.

It is clear that more studies on the factors limiting growth and photosynthesis in woody plants are needed. Since most available information indicates that deciduous trees are photosynthetically more efficient than conifers, their study seems a logical first step. Ledig (1969) has developed a mathematical model for relating growth and photosynthesis in tree seedlings. He includes rates of net photosynthesis, distribution of assimilate between the leaves and other organs, and seasonal aspects of photosynthesis. This type of approach appears to have considerable merit for evaluating the photosynthetic efficiency of a species.

Several workers have followed the seasonal course of photosynthesis, respiration, and metabolism in conifers (Gordon and Larson, 1968; Neish, 1958; Nelson, 1964; Schier, 1970; and Shiroya, et al., 1966). Few studies of this type have been undertaken with deciduous forest tree species. The study reported here was done to learn more

about the seasonal and developmental patterns of photosynthesis, respiration, and metabolism of labeled $^{14}CO_2$ in deciduous broad-leaved trees. The experimental plants were first-year potted seedlings of black walnut (Juglans nigra L.) and honeylocust (Gleditsia triancanthos L.) grown outdoors near East Lansing, Michigan. The following growth characteristics were studied at four dates from June to September, 1970: total height growth, leaf area accretion, and dry matter production of leaves, roots, and stem. The purpose of these measurements was to serve as an aid in the interpretation of measurements of photosynthesis, respiration, and translocation of ^{14}C labeled photosynthate.

Photosynthesis, dark respiration, and photorespiration also were measured at four dates from June to September, 1970 in order to study the seasonal development and efficiency of the photosynthetic processes.

Finally, the fate of labeled $^{14}CO_2$ was determined at four dates from June to September, 1970 in leaves, stem, and roots 24 hours after exposure and again at the time of leaf abscission to determine seasonal trends in metabolism of the two species.

CHAPTER I

HEIGHT GROWTH, LEAF AREA ACCRETION, AND DRY WEIGHT PRODUCTION

Introduction

A thorough understanding of growth and development patterns of a species is basic to the study of other physiological processes in that species. The course of seasonal height growth has been defined for several deciduous species (Kienholz, 1941; Kozlowski and Ward, 1957). Such studies have contributed to our understanding of the sympodial and preformed shoot growth habits of deciduous trees and shrubs. These studies also suggest possible translocation patterns and priority of stored foods metabolism. Other studies of seasonal cambial activity have also revealed general patterns for lateral growth of woody plants (Fritts, 1958; Reimer, 1949). Fayle (1968) has reviewed much of the literature on root growth.

Growth in one part of a tree is influenced by the growth in other parts. Such correlative growth responses are thought to reflect competition for food, water, minerals and hormones. An example is the ability of

reproductive structures to mobilize metabolic products often at the expense of vegetative organs. Little is known about the specific interactions of growth between the various organs of woody plants. Bey and Phares (1968) determined the seasonal growth patterns of the shoot and roots for five seed sources of one-year old black walnut and reported cumulative dry matter production of the roots of only about 10 grams. These low values coupled with an abrupt rather than sigmoid cumulative height growth curve seem to indicate that the seedlings in their study were grown under conditions of moisture stress.

The first objective of my study was to define normal seasonal development of first-year, potted black walnut and honeylocust seedlings grown outdoors under optimal conditions of moisture and mineral nutrition. Data are presented for cumulative height growth, leaf area accretion, and dry matter production of leaves, stem, and roots.

Materials and Methods

First-year black walnut (Juglans nigra L.) and honeylocust (Gleditsia triacanthos L.) seedlings were used as experimental material. Black walnuts were collected from several individual trees in the vicinity of Michigan State University in September and October, 1969. They were immediately husked, sown in buried 11½ liter pots

and left outdoors to overwinter at the M.S.U. Tree Research Center. Honeylocust seeds were collected from a single tree on the Michigan State University Campus in April, 1970 and sown in 4-liter pots on May 23, 1970 following treatment with concentrated sulfuric acid for 40 minutes. Germination of the black walnuts began on May 18, 1970 and continued sporadically through June 1970 until about 57 per cent of the seeds had germinated. Honeylocust began to germinate on June 10 and 99 per cent had germinated within a period of one week.

Pots containing germinated seeds were buried at a spacing of 61 x 61 cm behind a wind barrier at the Tree Research Center where they remained during the 1970 growing season. Each seedling was irrigated daily to maintain the soil in the pots at or near field capacity. At three-week intervals each pot was heavily fertilized with an aqueous solution containing major and minor nutrients. Frequent weeding kept the seedlings free of competition. The foliage of the walnut seedlings was sprayed several times with a 0.5 per cent aqueous solution of zineb to minimize damage from the walnut anthracnose [Gnomonia leptostyla (Fru.) Ces. and DeN.]. Temperature and solar radiation during the study period were not unusual for southern Michigan.

One month after germination 12 trees were selected from each species for height, leaf area, and dry weight determinations. Sample trees were selected on the basis of uniformity of height, appearance of the foliage, and

germination date. An additional 24 sample trees were selected on the same basis and were set aside for the photosynthesis and translocation portions of this study.

At four dates, approximately one month apart, three trees were selected at random from within the 12 sample trees and taken into the laboratory for analysis. Measurement dates for black walnut were June 15, July 27, August 17, and September 7 and for honeylocust July 13, August 10, September 1, and September 21. Total height measurements were recorded to the nearest 0.1 cm. Leaf areas were planimetered to the nearest 0.5 cm^2 and totaled for the whole seedling. Dry weights of leaves, stem, roots, and whole plant were determined by weighing to nearest 0.001 g after drying in an oven at 60°C for 24 hours.

Results and Discussion

Table 1 shows the seasonal changes in cumulative height, leaf area, and dry weight for each species during the 1970 growing season. Black walnut is typical of those deciduous species with the preformed shoot growth habit. Honeylocust shows a growth pattern characteristic of deciduous species with the sympodial growth habit where persistent terminal buds are not formed and the shoot tip dies and abscises.

TABLE 1.--Seasonal changes in cumulative height, leaf area, and dry weight of black walnut and honeylocust seedlings.

	Black Walnut			
	June 15	July 27	August 17	September 7
Cumulative ht (cm)	19.5[1]	26.3	26.8	26.7
Leaf area (cm^2)	52.6	124.4	84.4	52.5
Dry weight (g):				
Leaves	1.6	7.6	4.1	3.0
Stem	0.5	3.6	3.4	3.6
Roots	3.9	22.0	27.2	40.7
Whole Plant	6.0	33.2	29.1	47.3
Root/shoot ratio	1.8	2.0	3.7	6.2
	Honeylocust			
	July 13	August 10	September 1	September 21
Cumulative ht (cm)	22.7	35.7	36.7	37.0
Leaf area (cm^2)	13.5	24.2	25.9	35.8
Dry weight (g):				
Leaves	0.5	1.1	1.5	1.3
Stem	0.3	1.1	1.9	2.2
Roots	0.4	3.1	3.8	8.0
Whole plant	1.2	5.3	7.2	11.5
Root/shoot ratio	0.6	1.4	1.1	2.3

[1]Mean of 3 trees

Height Growth

Black walnut completed about 80 per cent of its height growth before June 15. Growth then slowed between June 15 and July 27. By July 27 height growth had ceased. Five black walnut sources grown at Ames, Iowa terminated height growth in early July (Bey and Phares, 1968).

Honeylocust seedlings showed a pattern of height growth characteristic of the sympodial growth habit. Only 60 per cent of its height growth had been attained by July 13. Growth continued at a reduced rate between July 13 and August 10 but was essentially complete by August 10. A small but significant amount of height growth continued through September 21.

Leaf Area Accretion

Leaf area increased very rapidly in the developing black walnut seedlings. It reached a level of 52.6 cm^2 by June 15 (Table 1). Between June 15 and July 27 the leaf surface area doubled and reached a peak of 124.4 cm^2. Between July 27 and August 17 leaf area decreased as older leaves of the lower crown began to senesce and abscise. Leaf abscission accelerated after August 17 and by September 27 only 52.5 cm^2 of leaf area remained.

Honeylocust seedlings showed a different pattern of leaf area accretion. Rapid leaf area buildup occurred during the first two months after germination. After August 10 leaf area accretion ceased for a short period.

Between September 1 and 21 a second increase in leaf area occurred and a maximum for the season of 35.8 cm^2 was reached. Leaves of lower crown began to abscise shortly before September 21.

Dry Weight Production

Trends in dry weight of the leaves were similar to those for leaf area accretion (Table 1). However, leaf dry weight decreased after September 1 while leaf area continued to increase. It is possible that development and enlargement of new leaves masked the abscission of some leaves in the lower crown.

Stem growth of black walnut occurred only in the first two months after germination. The most rapid increase occurred between June 15 and July 27. After July 27 stem growth ceased. Stem growth of honeylocust seedlings was greatest in early summer and fall. The most rapid rise occurred between July 13 and August 10. After September 1 a second peak period of stem growth increase was recorded.

Both species showed two periods of root growth separated by a period of relative inactivity (Table 1). In walnut seedlings the first peak of activity occurred between June 15 and July 27. Between July 27 and August 17 little root growth occurred. A second period of root growth occurred between August 17 and September 7. Most of the root growth in developing honeylocust seedlings

occurred between July 13 and August 10. As in walnut this was followed by a period of little growth. Between September 1 and September 27 a second period of root growth occurred.

Total plant dry matter reflected the activity of the root fractions (Table 1). Both species showed a steep rise in total dry matter followed by a period of inactivity. This in turn was followed by a second period of growth in the fall.

Comparison of Species

Developing black walnut and honeylocust seedlings showed height growth patterns characteristic of the preformed shoot and sympodial growth habits respectively. The height growth of black walnut occurred over a relatively short time period in comparison with honeylocust. The latter showed slight but continued height growth in the fall.

Both species have a compound leaf structure. Black walnut leaves are of the odd-pinnate type and honeylocust leaves are of the 2-pinnate decompound type. The pattern of leaf area accretion and the total leaf area produced in a single season were remarkably different between the two species. Walnut seedlings reached a maximum leaf area of 124.4 cm^2 within two months after germination and then leaf area declined. Walnut characteristically looses its leaves early in comparison to other deciduous species (Kramer, 1943). Most leaf area expansion

in honeylocust seedlings occurred within two months of germination. This was followed by a long period of little increase. A second period of leaf area buildup occurred in the fall. Perhaps the most remarkable difference between the two species was the tremendous difference in total leaf area produced. At its peak, relatively early in the growing season, the average black walnut seedling had a total leaf area of 124.4 cm^2. The maximum leaf area for honeylocust seedlings was 35.8 cm^2 and this was not obtained until September 21.

Much of the variation in early growth patterns and total dry matter between the two species may be due to the large difference in seed size. Black walnut is among the largest seeds of the plant kingdom, 40 seeds per pound while honeylocust seeds are considerably smaller, 2,800 seeds per pound (U. S. Forest Service, 1948).

Data on leaf dry matter generally followed the trends of leaf area accretion. Dry matter data for the stem and roots were further indicative of the preformed shoot and sympodial growth habits. Walnut stem growth ceased sometime prior to July 27 while honeylocust stem growth continued throughout the study period. Growth of the roots was maximal in early summer and fall. The results here agree partly with those of Lyr and Hoffmann (1967) who reported that maximum growth of roots of deciduous trees occurred in early summer compared with

coniferous species which grow more uniformly through the whole vegetative period.

CHAPTER II

PHOTOSYNTHESIS AND RESPIRATION

Introduction

Studies of photosynthesis of forest trees have dealt largely with single measures of photosynthesis under certain "standard" conditions. Other studies have been aimed at determining the effects of specific environmental factors on the photosynthetic process. Much of this work has been summarized by Larcher (1969) and Kramer (1958). Considerable effort has gone into the study of specific environmental factors which facilitate the optimum rate of photosynthesis in the species studied. Less attention has been given to the measurement of photosynthesis during developmental states within the annual cycles of growth and ontogeny of the species. Often age in years and plant size have been the only factors considered. It is usually not apparent to the reader just where within the annual cycle of growth and development the measurements were made.

Several studies have been made on seasonal fluctuations of photosynthesis in evergreen trees (Bourdeau, 1959;

Helms, 1965; McGregor and Kramer, 1963). Fewer studies have been made in deciduous trees (Heinicke and Childers, 1937; Saeki and Nomoto, 1958). Most of these studies were conducted outdoors and seasonal fluctuations in environmental factors obscure or complicate the study of photosynthetic efficiency.

In the work reported here photosynthetic efficiency for developing black walnut and honeylocust seedlings grown outdoors in southern Michigan was measured at intervals during the first growing season.

Materials and Methods

First-year black walnut and honeylocust seedlings grown outdoors under optimum conditions of soil moisture and mineral nutrition were used. See Chapter I for details of the experimental material.

On four dates, approximately one month apart from June to September 1970, different groups of five seedlings of each species were brought into the laboratory for determinations of photosynthesis and respiration rates under standard conditions of light intensity and temperature. Relative humidity was monitored but not controlled. Due to the mechanics of the translocation portion of the study, it was not possible to follow photosynthesis and respiration within the same group of five seedlings throughout the growing season. The sampling dates for walnut were June 15, July 27, August 17, and September 7. Honeylocust seedlings were sampled on July 13, August 10,

September 1, and September 21. The last date for each species coincided with the start of leaf abscission.

Photosynthesis and respiration measurements were made in a closed system using a controlled environment chamber constructed of acrylic plastic. The chamber was about 50 liters in volume and was surrounded by a water jacket for temperature stabilization. Four 500-watt weather resistant flood lamps were immersed in an acrylic plastic water bath above the chamber. Additional temperature and light control was achieved by placing the chamber into a large Sherer-Gillett controlled environment room. Light intensity was controlled with a Powerstat Type 3PN136B variable transformer. Temperature was stabilized at 25°C ±4 by varying the water levels in the water bath and chamber water jacket and manipulation of the temperature control of the controlled environment room. Relative humidity varied between 45 and 65 per cent.

A Beckman infrared gas analyzer model 215 and a Sevoriter recorder were used to detect and record CO_2 concentration changes. Small Rotron fans circulated air in the chamber and a Masterflex tubing pump maintained a flow rate of 900 ml/min. in the closed system. A "Drierite" (Ca SO_4) dessicant column was used to remove water vapor from the air stream flowing into the gas analyzer. Slight CO_2 absorption by the Ca SO_4 drying column did not significantly affect the results.

Individual seedlings including their pot container were placed into the acrylic chamber and equilibrated at 25°C and 10,000 ft-c of light intensity. Polyethylene bags were sealed around the pots to eliminate the effects of soil respiration and evaporation from the soil surface. After a 15-minute conditioning period the CO_2 concentration in the measurement chamber was raised to a level slightly in excess of 400 ppm, the chamber was sealed, and the CO_2 uptake was recorded until the chamber CO_2 concentration had decreased to around 200 ppm. The chamber was then opened to ambient air and the light intensity was lowered to 7,000 ft-c for honeylocust and 3,000 ft-c for black walnut. After a 15-minute conditioning period at the new light intensity the chamber was sealed and photosynthesis was again recorded from 400 ppm to 200 ppm. Upon reaching a CO_2 concentration of 200 ppm, the system was again opened to the ambient air and the light intensity was lowered a second time to 4,000 ft-c for honeylocust and 1,800 ft-c for black walnut. After a 15-minute conditioning period at the new light intensity the CO_2 uptake was recorded from 400 ppm to the CO_2 compensation point. Upon reaching the CO_2 compensation point the lights were turned off and dark respiration was recorded for two hours following the light period.

The rate of CO_2 depletion from 330 to 270 ppm was used as the measure of net photosynthesis. Rates were

computed from slopes of lines drawn tangent to the recorder tracings.

Results and Discussion

Photosynthetic Efficiency

Net photosynthesis in developing black walnut seedlings reached a peak incorporation of 7.8 mg CO_2 dm^{-2} hr^{-1} on July 27, the second measurement date (Figure 1). By August 17 net photosynthesis had declined to 7.1 mg CO_2 dm^{-2} hr^{-1}. After August 17 net photosynthesis decreased sharply to a low level of 1.3 mg CO_2 dm^{-2} hr^{-1} as rapid abscission began. A factorial analysis of net photosynthesis at the different measurement dates and light intensities revealed dates to be highly significant (Table 2). Dark respiration followed an irregular pattern during the period of study (Figure 1). The highest rates of dark respiration were found during the period of rapid leaf expansion and when leaf abscission began. The high rate on September 7 may reflect the dying of the leaves as they began to abscise.

Net photosynthesis in developing honeylocust seedlings showed a much different pattern (Figure 2). Net photosynthesis on July 13 was 10.6 mg CO_2 dm^{-2} hr^{-1}. By August 10 it had increased to 15.5 mg CO_2 dm^{-2} hr^{-1} and remained near this level through September 1. After September 1 net photosynthesis began to decline and by September 21 it was 12.2 mg CO_2 dm^{-2} hr^{-1}. The relatively

Figure 1.--Developmental changes in net photosynthesis, photorespiration and dark respiration in black walnut seedlings.

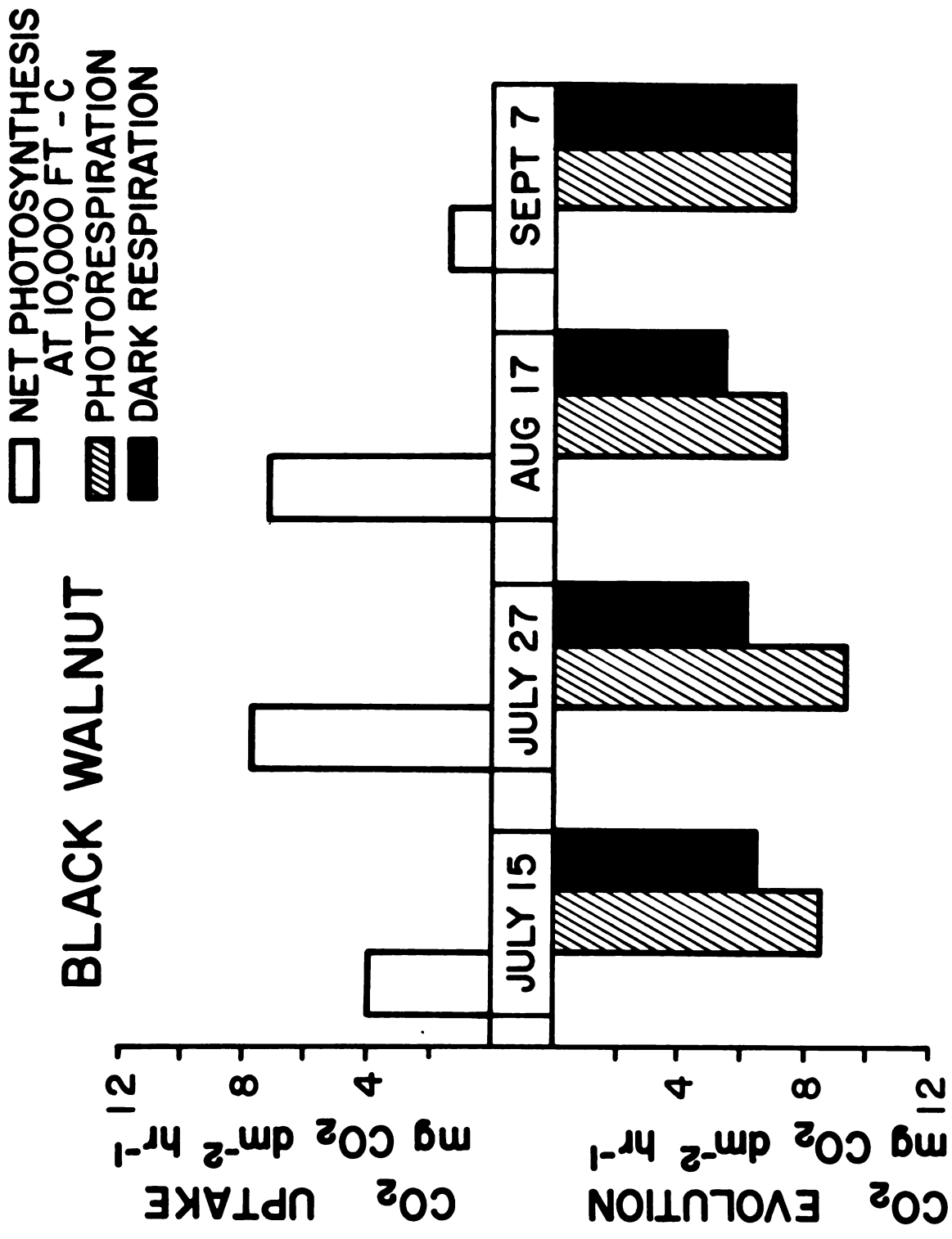

BLACK WALNUT
NET PHOTOSYNTHESIS AT 10,000 FT - C
PHOTORESPIRATION
DARK RESPIRATION
JULY 15
JULY 27
AUG 17
SEPT 7
CO2 UPTAKE
mg CO2 dm-2 hr-1
CO2 EVOLUTION
mg CO2 dm-2 hr-1
4
8
12

TABLE 2.--Analysis of variance of net photosynthesis of black walnut seedlings at four dates[1] and three light intensities.[2]

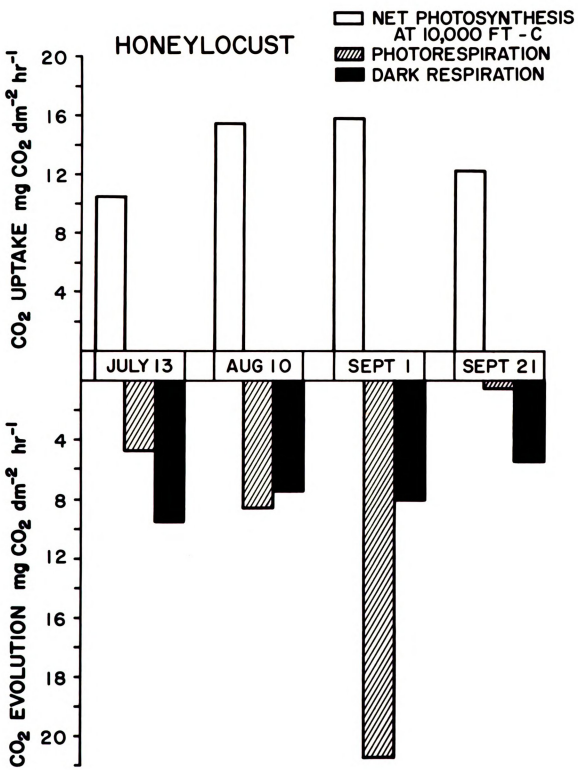
Source of Variance	Percent of Total Variance
Date	87**
Light intensity	8**
Date x light intensity	1
Error	4

[1]June 15, July 27, August 17, and September 7

[2]1,800; 3,000; and 10,000 ft-c

**Significant at the 1% level

Figure 2.--Developmental changes in net photosynthesis, photorespiration, and dark respiration in honeylocust seedlings.


HONEYLOCUST
NET PHOTOSYNTHESIS AT 10,000 FT - C
PHOTORESPIRATION
DARK RESPIRATION
CO2 UPTAKE mg CO2 dm-2 hr-1
CO2 EVOLUTION mg CO2 dm-2 hr-1
20
16
12
8
4
4
8
12
16
18
20
JULY 13
AUG 10
SEPT 1
SEPT 21

high rate of net photosynthesis on September 21 may be characteristic of the sympodial growth habit. The development of new leaves may have kept the rate of net photosynthesis at a high level even though leaves of the lower crown had begun to abscise. A factorial analysis of net photosynthesis of the different measurement dates and light intensities showed date to be highly significant (Table 3). Dark respiration was highest, 9.5 mg CO_2 dm^{-2} hr^{-1}, in honeylocust seedlings on July 13 as rapid leaf expansion occurred (Figure 2). On August 10 it declined to approximately 8 mg CO_2 dm^{-2} hr^{-1} and remained near this level through September 1. In sharp contrast to black walnut dark respiration in honeylocust was lowest as leaf abscission began.

There was considerable variation in rates of net photosynthesis and dark respiration among individual trees of both species. Net photosynthesis ranged as high as 14 mg CO_2 dm^{-2} hr^{-1} for black walnut and as high as 20 mg CO_2 dm^{-2} hr^{-1} for honeylocust (see Appendix, Table 10). Rates of CO_2 evolution for dark respiration were as high as 10 mg CO_2 dm^{-2} hr^{-1} for black walnut and 13 mg CO_2 dm^{-2} hr^{-1} for honeylocust (see Appendix, Table 11).

Both black walnut and honeylocust are considered shade intolerant and reproduction is limited to openings in the forest canopy (Baker, 1949). The low rate of net photosynthesis of black walnut in comparison with honeylocust was unexpected. The growth of walnut seedlings is

TABLE 3.--Analysis of variance of net photosynthesis of honeylocust seedlings at four dates[1] and three light intensities.[2]

Source of Variance	Percent of Total Variance
Date	65**
Light intensity	25**
Date x light intensity	1
Error	9

[1]July 13, August 10, September 1, and September 21

[2]4,000; 7,000; and 10,000 ft-c

**Significant at 1% level

remarkably rapid considering its early leaf abscission in comparison with other hardwoods. There is no available information on the early growth rates of honeylocust seedlings. To further investigate the differences between the two species, rates of total photosynthesis were determined on a leaf area and whole plant basis (Table 4). On a leaf area basis honeylocust seedlings exhibited a remarkable superiority in carbon dioxide uptake over walnut. However, when total photosynthesis was expressed on a whole plant basis the situation was reversed. Black walnut presumably because of its greater leaf surface area surpassed honeylocust in total CO_2 uptake. It is possible other leaf factors also may be involved. It seems reasonable that differences in the number, distribution, and size of stomata as well as the thickness of the leaf and its mesophyll may further contribute to differences in photosynthesis between the two species. In walnut stomates are restricted to the upper epidermis and average 46,000 per cm^2 (Meyer and Anderson, 1952). Observations by the author indicate that the stomates of honeylocust also are restricted to the upper epidermis. Data on stomatal size and relative distribution are not available for either species.

Effect of Light Intensity

The limited objectives of this study did not permit a detailed examination of the effects of light intensity on the photosynthetic apparatus. Table 5 shows the effects of

TABLE 4.--Seasonal changes in total photosynthesis[1] of developing black walnut and honeylocust seedlings at 10,000 ft-c.

Species and Dates	Total Photosynthesis	
	mg CO_2 dm^{-2} hr^{-1}	mg CO_2 $Seedling^{-1}$ hr^{-1}
Black Walnut		
June 15	10.4[2]	53.8
July 27	12.8	164.5
August 17	12.7	108.0
September 7	8.4	34.1
Honeylocust		
July 13	20.0	25.6
August 10	23.0	55.6
September 1	23.9	60.2
September 21	19.3	65.4

[1]Total photosynthesis = net photosynthesis + dark respiration

[2]Mean of 5 trees

TABLE 5.--Developmental changes in net photosynthesis at different light intensities in black walnut and honeylocust seedlings.

Species and Date	Net Photosynthesis[2]		
Black Walnut	1,800 ft-c	3,000 ft-c	10,000 ft-c
		mg CO_2 dm^{-2} hr^{-1}	
June 15	4.4 a	5.0 a b	4.0 a b
July 27	5.9 a	8.8 a	7.8 a
August 17	5.0 a	6.8 a	7.1 a
September 7	1.4 a	1.6 b	1.3 b
Honeylocust	4,000 ft-c	7,000 ft-c	10,000 ft-c
		mg CO_2 dm^{-2} hr^{-1}	
July 13	10.9 a	12.4 a	10.6 a
August 10	14.3 a	16.6 a	15.5 a
September 1	14.1 a	16.8 a	15.8 a
September 21	10.3 a	13.4 a	12.2 a

[1]Means not followed by same letter are significantly different at 5 per cent level (Tukey's ω-procedure)

[2]Mean of 5 trees

three light intensities on net photosynthesis in walnut and honeylocust. Factorial analyses showed highly significant differences in the effects of light intensity (Tables 2 and 3). In both species there was an increase in net photosynthesis between the low and intermediate light intensities. The inability of honeylocust to carry on net photosynthesis at light intensities below 4,000 ft-c early in the annual growth cycle, did not permit the study of the same light intensities for both species. There was a decline in net photosynthesis at full sunlight, 10,000 ft-c, for both species. Other workers have found similar solarization effects in other deciduous hardwoods (Borman, 1953; Kozlowski, 1949). Solarization in conifers occurs at much higher light intensities (Ronco, 1961).

The results of this study permit only some very general statements concerning light saturation for each species. Light saturation for black walnut lies somewhere between 3,000 and 10,000 ft-c. It is probably closer to 3,000 ft-c than 10,000 ft-c because light saturation for other hardwoods with similar growth habits lies in this range. Honeylocust has an unexpectedly high light saturation. It lies somewhere between 7,000 and 10,000 ft-c. Early in the growing season no measurable net photosynthesis occurred in this species below 4,000 ft-c. Later net photosynthesis was detected at lower light intensities.

Photorespiration

The question has often been asked, do plants respire in the light or is "dark" respiration turned off during active photosynthesis? From measurements of the rates of O_2 and CO_2 exchange in illuminated plants it has been determined that most plants do respire in the light while they are carrying on photosynthesis. This photorespiration is separate from mitochondrial respiration since it is not sensitive to inhibitors of mitochondrial respiration. Photorespiration consumes reducing power generated by photosynthesis and uses it to reduce molecular oxygen. Photorespiration thus shortcircuits photosynthesis by diverting the normal flow of light-induced reducing power from the reduction of CO_2 to the reduction of O_2. It has been estimated that in some plants photorespiration utilizes up to 50 per cent of the reducing power generated by photosynthesis (Lehninger, 1970). Some tropical plants including maize and sugar cane show no apparent photorespiration.

Photorespiration is difficult to measure since it involves simultaneous CO_2 exchange with photosynthesis. Photorespiration has seldom been measured in forest trees. Townsend (1969) found rates of photorespiration in young western white pine seedlings approaching those of dark respiration. In certain crop plants the release of CO_2 in photorespiration may be three to five times greater than the rate of release in darkness (Zelitch, 1969). No data

is available on photorespiration measurement in deciduous hardwoods.

Photorespiration in this study was estimated by extrapolating photosynthetic response curves to zero CO_2 concentration (Brix, 1968). The accuracy of this technique has been challenged (Bravdo, 1968; Brix, 1968; Zelitch, 1966). However, its use seems justified since little photorespiration data are available for forest trees and since all current techniques for estimating photorespiration have been questioned.

Seasonal trends in photorespiration derived by the extrapolation method are shown in Figures 1 and 2. It is evident that photorespiration does change substantially within the annual growth cycle of plants. Rates of photorespiration were generally related to photosynthetic activity. High rates of photorespiration usually accompanied high rates of net photosynthesis. Rates of photorespiration were considerably higher in honeylocust than black walnut.

There was considerable variation in rates of photorespiration among individual trees of both species. Photorespiration ranged as high as 9 mg CO_2 dm^{-2} hr^{-1} in black walnut and 22 mg CO_2 dm^{-2} hr^{-1} for honeylocust (see Appendix, Table 12).

The rate of photorespiration has been shown to increase with increasing light intensity. This occurred in black walnut on three dates (Table 6). However,

TABLE 6.--Effect of developmental stages on photorespiration at different light intensities in black walnut and honeylocust seedlings.

Species and Date	Photorespiration	
Black Walnut	3,000 ft-c	10,000 ft-c
	mg CO_2 dm^{-2} hr^{-1}	
June 15	6.4[1]	8.4
July 27	7.6	9.2
August 17	7.9	7.3
September 7	4.0	7.6
Honeylocust	4,000 ft-c	10,000 ft-c
	mg CO_2 dm^{-2} hr^{-1}	
July 13	8.8	4.8
August 10	10.1	8.4
September 1	20.1	21.5
September 21	4.7	0.6

[1]Mean of 5 trees

increasing the light intensity from 4,000 to 10,000 ft-c caused a decrease in the rate of photorespiration in honeylocust on three dates. The reason for this is unknown. There was a measurable decrease in the rate of net photosynthesis at 10,000 ft-c. Perhaps this decrease resulting from solarization would also show up in decreased photorespiration since photosynthesis and photorespiration are biochemically linked or because certain cellular components of the photorespiratory process are affected. A similar decrease in photosynthetic activity in black walnut did not result in decreased photorespiration.

CO_2 Compensation Point

Another means of evaluating the photosynthetic efficiency of plants is the determination and comparison of CO_2 compensation points. The CO_2 compensation point may be defined as that concentration of CO_2 at which the evolution of CO_2 by respiration from illuminated leaves equals photosynthetic CO_2 uptake. Low compensation points are thought to be correlated with high photosynthetic efficiency because plants lacking photorespiration have compensation points near 0 ppm CO_2.

Few determinations of the CO_2 compensation point have been made for forest trees. Townsend (1969) found extremely high CO_2 compensation points in young western white pine seedlings. The CO_2 compensation points determined in this study were similar for the two species

(Table 7). The compensation points were generally indicative of photosynthetic activity. For both species the CO_2 compensation points were lowest when rates of net photosynthesis were highest. Later, as photosynthetic activity decreased the compensation points increased.

There was considerable variation in CO_2 compensation points among individual trees of both species. Compensation points ranged as low as 95 ppm for black walnut and 105 ppm for honeylocust during the period when rates of photosynthesis were highest (see Appendix, Table 13).

TABLE 7.--Effect of developmental stages on the CO_2 compensation point in black walnut and honeylocust seedlings at 25°C.

Species and Date	CO_2 Compensation Point ppm
Black Walnut	
June 15	160[1]
July 27	118
August 17	145
September 7	232
Honeylocust	
July 13	155
August 10	114
September 1	156
September 21	[2]

[1]Mean of 5 trees

[2]Data not obtained

CHAPTER III

TRANSLOCATION OF LABELED PHOTOSYNTHATE

Introduction

Many of the great advances in biochemistry during the last decade were made possible by the use of radioisotopes. A major contribution of the radiotracer technique was the determination of the path of carbon in photosynthesis by Calvin and Benson (1948). Radioactive carbon has been used to study seasonal photosynthate production and translocation in conifers (Gordon and Larson, 1968; Schier, 1970; Shiroya et al., 1966). Fewer studies have followed the rate of $^{14}CO_2$ in deciduous hardwoods (Hanson, 1964). Roberts (1964) used $^{14}CO_2$ to study the effect of water stress on the translocation of photosynthate in yellow-poplar (Liriodendron tulipifera L.). More recently Larson and Gordon (1969) used $^{14}CO_2$ to study the translocation of photosynthate from selected leaves of young cottonwood (Populus deltoides Bartr.) trees.

Most metabolic studies of deciduous trees have been limited in that they have been of short duration and usually have not attempted to follow the incorporation of

^{14}C into specific compounds extracted from various plant organs. In this study developing black walnut and honeylocust seedlings grown outdoors were photosynthetically labeled at several dates during the growing season. At two time intervals following labeling the radioactivity in specific compounds of leaf, stem, and root fractions was determined.

Materials and Methods

First-year black walnut and honeylocust seedlings grown outdoors under optimum conditions of soil moisture and mineral nutrition were used as experimental material. See Chapter I for details about the experimental material.

On four dates, approximately one month apart from June to September 1970, six seedlings of each species were brought into the laboratory and exposed to $^{14}CO_2$ under defined conditions of light intensity and temperature. Walnut seedlings were labeled on June 15, July 27, August 17, and September 7. Honeylocust seedlings were labeled on July 13, August 10, September 1, and September 21.

Whole seedlings were labeled in the same type of closed system used for the determination of rates of photosynthesis and respiration described in Chapter II.

Individual seedlings including the pot container were placed in the acrylic chamber at a temperature of 25°C and a light intensity of 10,000 ft-c. Polyethylene bags were used to seal off the pots. After a 15-minute

conditioning period the chamber was sealed off and the plant was exposed to about 500 μc of $^{14}CO_2$ by reacting $Ba^{14}CO_3$ with perchloric acid. All plants were exposed to $^{14}CO_2$ for a period of two hours. On most dates this was sufficient time to reach the CO_2 compensation point. Immediately after labeling the plants were returned to the outdoors.

One day after photoassimilation three of the labeled plants were separated from the soil by washing and divided into leaves, stem, and roots. The remaining three seedlings were harvested in similar fashion on September 21. This date will hence be referred to as the end of the growing season as it coincided with the start of rapid leaf abscission. Each plant part was cut into small pieces and separated into five fractions, amino acids, organic acids, sugars, non-water soluble, and ethanol insoluble, by means of extraction procedure shown in Figure 3. Ion exchange columns were of the type used by Romberger (1960).

The non-water soluble and ethanol insoluble fractions were taken to dryness. Sugar, amino acid, and organic acid fractions were reduced to about 5 ml. To determine radioactivity, each fraction was wet-combusted (Van Slyke et al., 1951) and the evolved $^{14}CO_2$ was trapped in a solution of ethanolamine and glycol monomethyl ether (1:2 v/v). The scintillation solution was prepared according to the method of Jaffay and Alvarez (1961). Radioactivity was determined in a Packard Tricarb Liquid

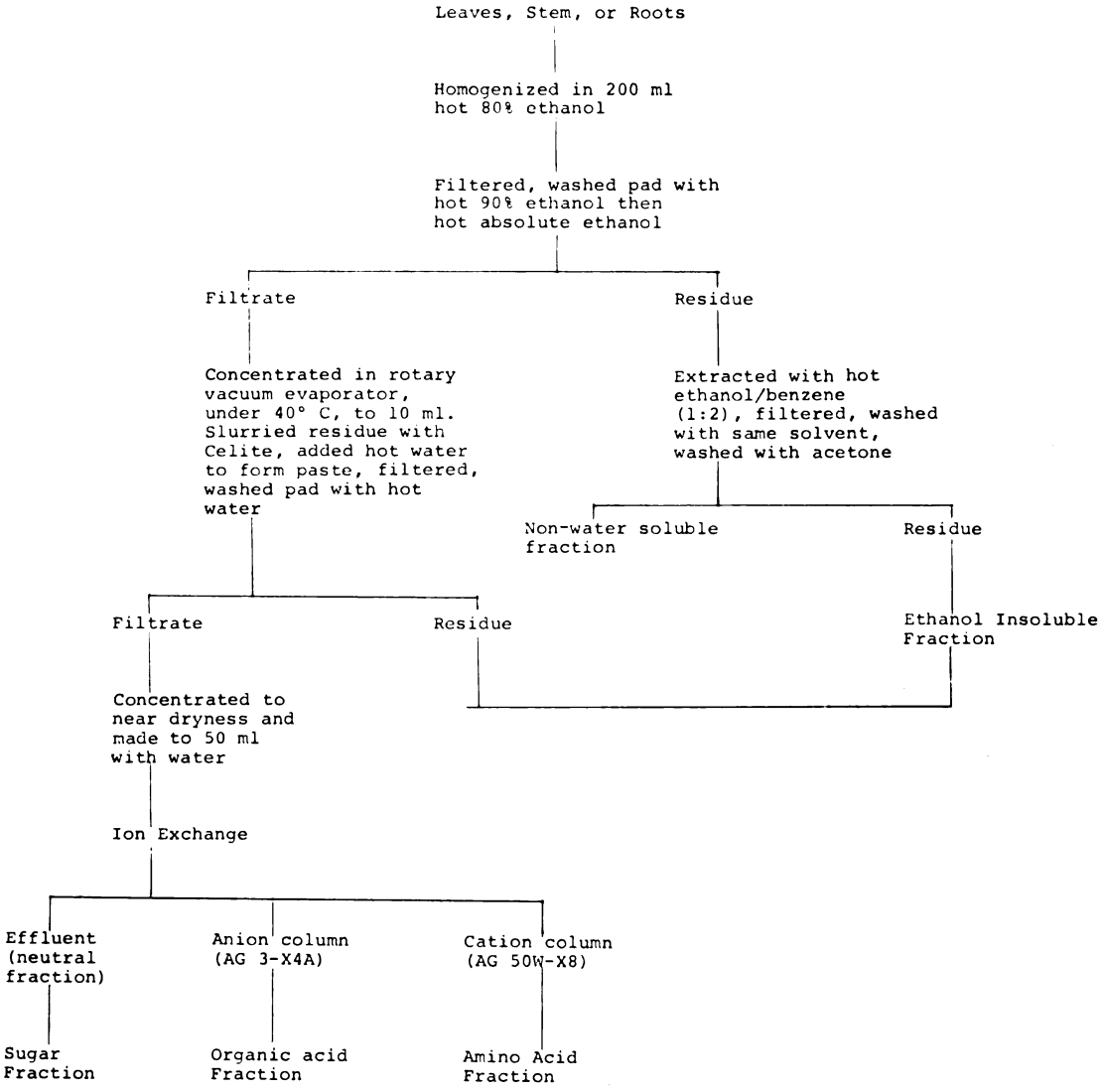


Figure 3.--Extraction procedure.

Scintillation Spectrometer. After quench correction by the internal standard method, radioactivity of all fractions in each plant part was expressed as a percentage of the total DPM in the whole tree at the date of harvest. Quantitive determinations of the various fractions were not made and therefore specific activity could not be calculated.

Results and Discussion

Short Term Translocation of ^{14}C in Black Walnut

Black walnut seedlings harvested one day after photoassimilation of $^{14}CO_2$ at several growth stages revealed a pattern of metabolism and translocation which may be characteristic of trees with the preformed shoot growth habit. Developing organs and meristematic areas are known to be sinks for photosynthates. See Appendix, Table 14 for a complete listing of the incorporation of ^{14}C into the various compounds studied.

Between June 15 and July 27 there was a very large increase in the leaf area of the walnut seedlings. This growth pattern was reflected in the high levels of radioactivity recovered from compounds extracted from the leaves (Figure 4). Approximately 79 per cent of the total activity recovered on June 15 was found in the leaves (Table 8). A high proportion of this activity occurred in the non-water soluble fraction, probably in chlorophyll and other pigments (Figure 4). The other ethanol-soluble compounds, sugars and amino acids, were at relatively high

Figure 4.--Seasonal effect on incorporation of ^{14}C into major fractions extracted from leaves, stem, and roots of black walnut seedlings one day after treatment.

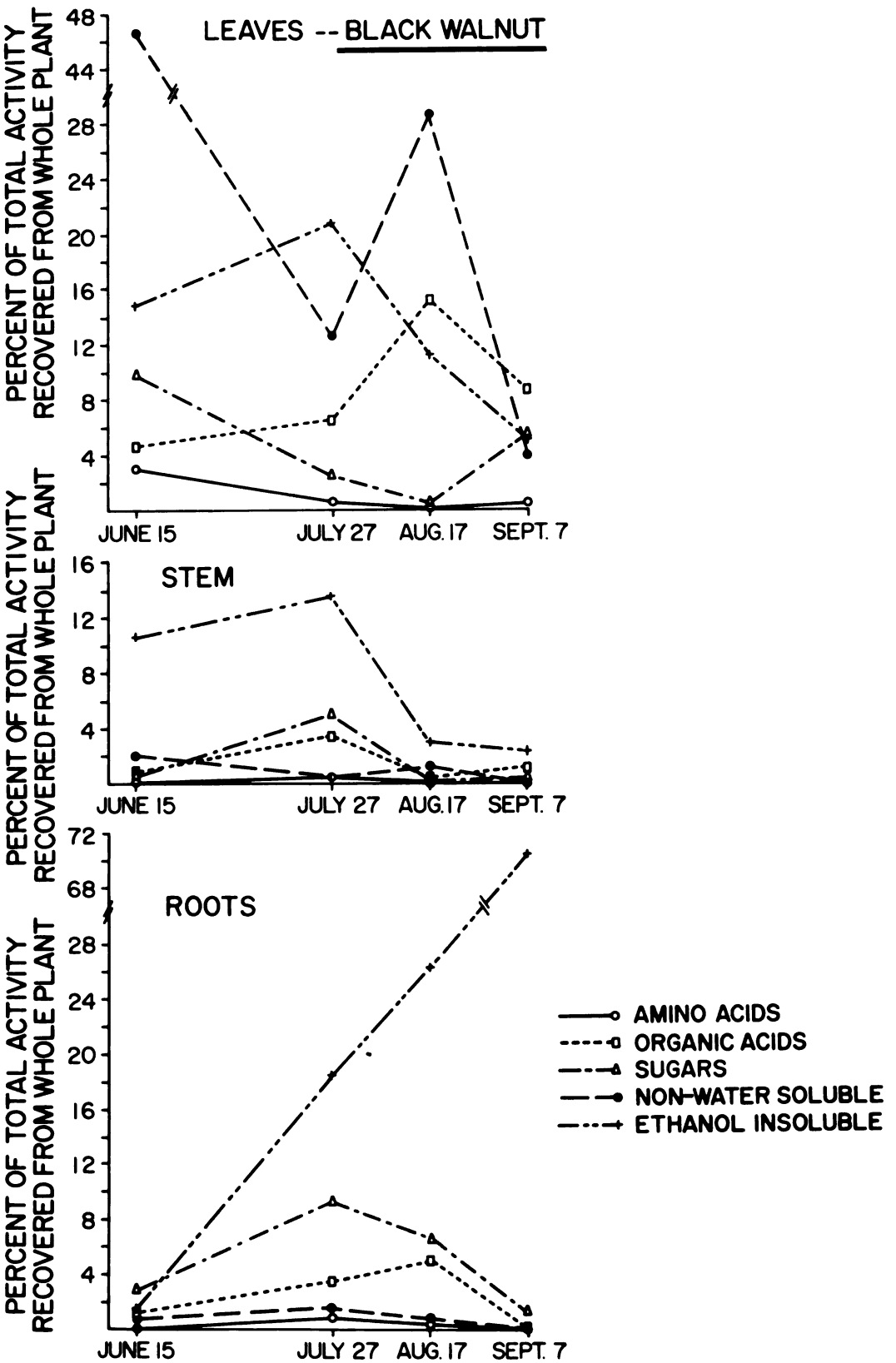

LEAVES -- BLACK WALNUT
STEM
ROOTS
PERCENT OF TOTAL ACTIVITY RECOVERED FROM WHOLE PLANT
JUNE 15
JULY 27
AUG. 17
SEPT. 7
AMINO ACIDS
ORGANIC ACIDS
SUGARS
NON-WATER SOLUBLE
ETHANOL INSOLUBLE

TABLE 8.--Redistribution of ^{14}C between the leaves, stems, and roots of black walnut and honeylocust seedlings.

Species and Date	Harvested after one day			Harvested at end of growing season		
	Leaves	Stem	Roots	Leaves	Stem	Roots
	----------Per cent of total activity----------					
Black Walnut						
Labeled on:						
June 15	79.2[1]	14.4	6.4	14.5	16.0	69.5
July 27	43.3	23.0	33.7	21.3	12.2	66.5
August 17	55.8	5.0	39.2	18.3	8.5	73.2
September 7	24.0	4.0	72.0	26.4	10.7	62.9
Honeylocust						
Labeled on:						
July 13	49.8	19.5	30.7	23.6	28.3	48.1
August 10	60.4	20.7	18.9	44.2	30.0	25.8
September 1	45.8	41.4	12.8	42.9	22.5	34.6
September 21	10.9	29.0	60.1	34.4	27.8	37.8

[1]Mean of 3 trees

levels on this date also. Radioactivity in the ethanol-insoluble fraction was considerably higher than that found in conifers after similar short term periods of assimilation. This was probably due to the incorporation of ^{14}C into the construction of cell walls and other structural compounds in leaf development. Further evidence that the leaves were the principal center of metabolic activity on June 15 was obtained by comparing them with levels of radioactivity extracted from the stem and roots (Figure 4). Radioactivity in the stem and root fractions were relatively low on June 15. A significant amount of radioactivity was found in the ethanol-insoluble fraction of the stem. This was undoubtedly due to the rapid primary and secondary growth which took place at this time. Radioactivity in the ethanol soluble and insoluble fractions of the roots was at a low level on June 15.

By July 27 metabolic activity in the stem and roots increased. Leaf area reached its maximum by July 27 and this was reflected in the increased radioactivity of the ethanol-insoluble fraction which peaked at this time. The non-water soluble fraction of the leaves had decreased by July 27, probably indicative of reduced pigment synthesis. The level of radioactivity in most fractions of the stem reached a peak on July 27 indicative of the cessation of primary growth. The level of radioactivity present in the stem after July 27 probably was due to lateral growth and lignification.

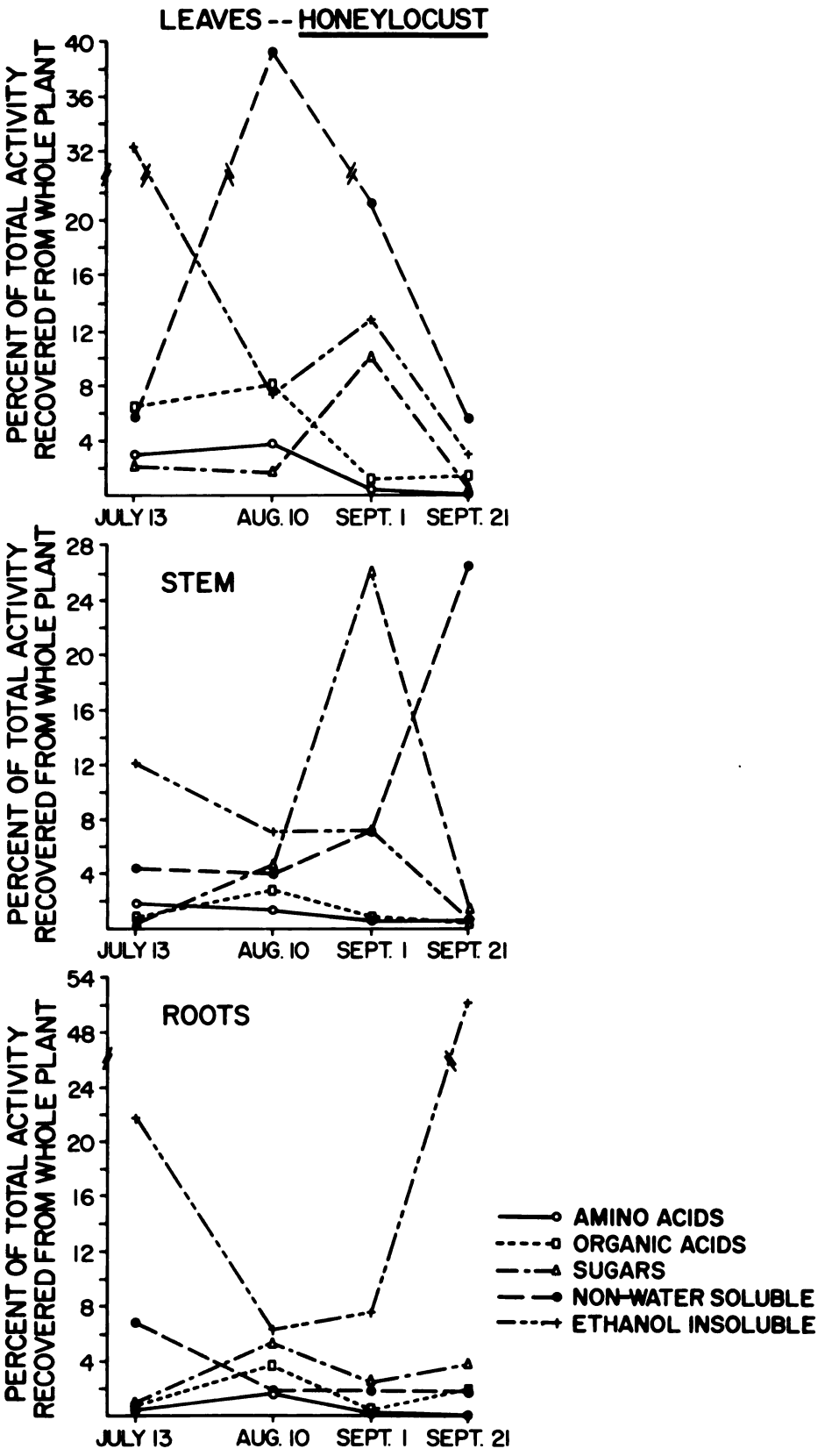
After July 27 the center of metabolic activity moved from the stem to the roots. The amount of radioactivity recovered from the ethanol-insoluble fraction of the roots during the growing season increased almost linearly (Figure 4). On June 15 only 6 per cent of the total radioactivity was recovered from the roots (Table 8). By September 7 this fraction accounted for 72 per cent of the activity. Other root extracts reached a peak between July 27 and September 7. By September 7 all root fractions except the ethanol-insoluble one had decreased to low levels similar to those found on June 15.

Short Term Translocation of ^{14}C in Honeylocust

Honeylocust seedlings harvested one day after $^{14}CO_2$ photoassimilation did not show a distinct pattern of shifting metabolic activity from the leaves to the stem and finally to the roots (Figure 5). This indicates that metabolic and translocation patterns in deciduous plants differ between the sympodial and preformed shoot habits. A complete listing of the incorporation of ^{14}C into the various compounds studied is given in the Appendix, Table 15.

There was considerable radioactivity in the leaves of honeylocust on July 13 but measurable amounts of radioactivity also were extracted from the stem and roots (Table 8). Much of the radioactivity extracted from the leaves on July 13 was found in the ethanol-insoluble fraction probably reflecting leaf expansion. The

Figure 5.--Seasonal effect of incorporation of ^{14}C into major fractions extracted from leaves, stem, and roots of honeylocust one day after treatment.


LEAVES -- HONEYLOCUST
PERCENT OF TOTAL ACTIVITY RECOVERED FROM WHOLE PLANT
40
36
32
20
16
12
8
4
JULY 13
AUG. 10
SEPT. 1
SEPT. 21
STEM
PERCENT OF TOTAL ACTIVITY RECOVERED FROM WHOLE PLANT
28
24
20
16
12
8
4
JULY 13
AUG. 10
SEPT. 1
SEPT. 21
ROOTS
PERCENT OF TOTAL ACTIVITY RECOVERED FROM WHOLE PLANT
54
48
24
20
16
12
8
4
JULY 13
AUG. 10
SEPT. 1
SEPT. 21
AMINO ACIDS
ORGANIC ACIDS
SUGARS
NON-WATER SOLUBLE
ETHANOL INSOLUBLE

ethanol-insoluble fraction also accounted for most of the radioactivity recovered from the stem and roots, probably indicative of the rapid primary growth which occurred at this time.

After July 13 much of the radioactivity in the leaf and stem portions was concentrated in the several ethanol-soluble fractions. These fractions in the leaves remained at high levels through September 1. This indicated that metabolic activity continues at relatively high levels going into the fall months. Between July 13 and August 10 there was a decrease in the amount of radioactivity recovered from the ethanol-insoluble fraction of the leaves. After August 10 the large portion of the radioactivity in the ethanol-insoluble fraction of the stem was probably indicative of secondary growth.

The roots of honeylocust had two periods of active growth. The first occurred at the start of the growing season and was followed by a period of relative inactivity. A second surge of growth occurred near the end of the growing season. Metabolic activity showed a similar pattern as evidenced from the radioactivity recovered from the ethanol-insoluble fractions of the roots (Figure 5). Between August 10 and September 1 little ^{14}C was incorporated in the ethanol-insoluble fraction of honeylocust roots.

Redistribution of ^{14}C in Black Walnut

The harvesting of plants at the end of the growing season after labeling at several points during the period of growth and development provided some valuable insight on the redistribution of metabolic compounds within deciduous species as the leafless period begins.

As the end of the growing season approached, radioactivity in the roots accounted for about 65 to 70 per cent of the total radioactivity recovered from walnut seedlings (Table 8). Much of this activity was found in the ethanol-insoluble fraction. Apparently this reflects the conversion of metabolities into storage compounds and the continuation of root growth into the fall months.

Only 5 to 10 per cent of the total radioactivity was recovered from the leaves as leaf abscission began (Table 8). This is in contrast to higher levels found a short time after labeling. Thus it appears that walnut was able to transport many of the labeled metabolities out of the leaves as abscission began.

The stem contained about 10 per cent of the recovered radioactivity at the end of the growing season. Similar levels were found during the growth season after short term assimilation. A high proportion of this activity was found in ethanol insoluble compounds and probably reflects the incorporation of ^{14}C into non-mobile structural compounds.

Redistribution of ^{14}C in Honeylocust

Less redistribution of ^{14}C occurred in honeylocust as the growing season ended than in walnut. A large amount of ^{14}C, 34 per cent, remained in the leaves on September 21 (Table 8). Much of this activity was located in ethanol-soluble compounds indicating that high metabolic activity continues in the sympodial growth habit as leaf abscission begins. The extremely low activity, 3 per cent, in the ethanol-insoluble fraction of the leaves on September 21 was due to a shorter time interval between labeling and harvesting.

The Incorporation of ^{14}C into the Sugars of Black Walnut

Subsamples were taken from the sugar extracts of black walnut seedlings labeled on July 27 and September 7 and harvested the following day to study the incorporation of ^{14}C into specific sugars. These dates were chosen to compare periods of high and low photosynthetic activity.

The neutral fraction from ion exchange (Figure 3) containing sugars was reduced to 1 ml and chromotographed on Whatman # 1 paper with butanol-water-acetic acid (4:5:1) in the descending direction for 48 hours. Autoradiography was used to locate the sugars. Tentative identification was established by comparing R_G's with those obtained in other species under similar chromatographic conditions. Standard sized discs containing the various labeled sugars were cut from the chromatograms

and placed in vials containing 15 ml of the scintillation medium used in other parts of this study. After a 24-hour equilibration period the vial containing the chromatograph disc was placed in a Packard Tricarb Scintillation Spectrometer for determination of radioactivity. Previous work has shown that counting efficiencies on the order of 85 per cent can be obtained by this technique (Davidson, 1962).

Table 9 shows the results of this analysis. A period of maximum photosynthesis, June 15, is compared with September 7, a period of low photosynthetic activity. Sucrose accounted for 66 per cent of the total radioactivity recovered on June 15. This was expected as sucrose is believed to be the principal translocated form of carbohydrate. Much of the sucrose was recovered from the roots indicating rapid translocation of photosynthates. Smaller amounts were found in the leaves and stem. Some glucose and fructose were labeled. Very small amounts of ^{14}C were found in raffinose and stachyose in the leaves only. Little ^{14}C was recovered from walnut stems.

On September 7 the sucrose fraction contained only 44 per cent of the total ^{14}C recovered from walnut seedlings. Increased amounts of radioactivity were found in the glucose and fructose fractions. Most of this activity was found in the leaves probably indicating that most $^{14}CO_2$ fixed on September 7 had remained in the leaves and translocation had slowed. Zimmermann (1968) found little phloem translocation and increased levels of sucrose in

TABLE 9.--Changes in the distribution of ^{14}C among the sugars of leaves, stem, and roots of black walnut seedlings on two dates during the first growing season.

	Percent of total ^{14}C in the sugar fraction after 24 hours of assimilation	
	June 15	September 7
	Sucrose	
Leaves	15.6	16.5
Stem	6.2	3.2
Roots	44.1	24.6
	Glucose	
Leaves	9.1	19.2
Stem	0	0.5
Roots	9.6	6.7
	Fructose	
Leaves	7.6	18.7
Stem	0	0.5
Roots	6.5	6.5
	Raffinose	
Leaves	0.8	0.9
Stem	0	0
Roots	0	1.6
	Stachyose	
Leaves	0.5	0.4
Stem	0	0
Roots	0	0.7
	All	
Leaves	33.6	55.7
Stem	6.2	4.2
Roots	60.2	40.1

[1]Mean of 3 trees

the leaves of white ash (Fraxinus americana L.) trees following leaf senescence. Increased radioactivity was recovered in the stachyose and raffinose fractions on September 7, particularly from the roots. Raffinose and stachyose are common in later phases of the growing season and in perennial plants and their buildup may be associated with cold-hardiness (Axelrod, 1965).

CHAPTER IV

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Terminal buds are formed by many but not all deciduous hardwoods. In the preformed shoot type of growth the foliage leaf primordia and their unextended internodes constitute the preformed shoot which will be extended during the next growth period. Some deciduous species do not form terminal buds. These species are said to exhibit the sympodial growth habit. In these species the shoot tip aborts in summer and the function of the terminal bud is assumed by the uppermost laterals.

An important contribution of this study was the comparison of growth patterns and photosynthetic efficiency between these two contrasting types of growth. Black walnut is typical of many species which exhibit the preformed shoot growth habit while honeylocust is characteristic of those with the sympodial growth habit.

Height growth of black walnut was of short duration and was completed early in the growing season. In contrast honeylocust seedlings grew continuously to September 1. Similar trends were found in the dry matter production

of the leaves and stem. However, root growth in both species continued through the summer to September 21. The patterns of leaf area accretion and the total leaf area produced in a single season were remarkably different between the two species. Walnut seedlings reached a maximum leaf area of about 124 cm^2 within two months of germination and then leaf area rapidly declined due to abscission. Most leaf area accretion in honeylocust seedlings also occurred within two months of germination, but some new leaves were added continuously through the growing season.

Honeylocust seedlings exhibited a remarkable superiority in CO_2 uptake over black walnut seedlings on a leaf area basis. However, black walnut because of its larger leaf area had much higher rates of CO_2 uptake on a whole seedling basis. Net photosynthesis increased gradually during the summer in honeylocust seedlings and reached a peak incorporation of 15.5 mg CO_2 dm^{-2} hr^{-1} on August 10 and remained at a high level through September 21 as leaf abscission began. Net photosynthesis in black walnut reached a peak of 7.8 mg CO_2 dm^{-2} hr^{-1} on July 27 and then declined sharply to a low level of 1.3 mg CO_2 dm^{-2} hr^{-1} on September 7. There was considerable variation in rates of net photosynthesis, dark respiration, and photorespiration among individual trees of both species. Net photosynthesis ranged as high as 14 mg CO_2 dm^{-2} hr^{-1} in black walnut. A high of 20 mg CO_2 dm^{-2} hr^{-1} was

recorded for honeylocust. Rates of dark respiration were as high as 10 mg CO_2 dm^{-2} hr^{-1} for black walnut and 13 mg CO_2 dm^{-2} hr^{-1} for honeylocust. Photorespiration ranged as high as 9 mg CO_2 dm^{-2} hr^{-1} in black walnut and a high of 22 mg CO_2 dm^{-2} hr^{-1} was recorded for honeylocust. Limited data indicated that the light saturation point for honeylocust was near 7,000 ft-c while that for black walnut was substantially lower, being approximately 3,000 ft-c. Rates of photorespiration and the CO_2 compensation points differed greatly during the annual cycle of growth and were indicative of photosynthetic activity. For both species the CO_2 compensation points were lowest when rates of net photosynthesis were highest. Later, as photosynthesis activity decreased the compensation points increased.

There were clear differences between the two species in patterns of metabolism and translocation of photosynthate. Black walnut seedlings shifted their metabolic activity from the leaves to the stem and finally to the roots as the growing season progressed. In the fall practically all metabolic activity was found in the roots. In contrast to black walnut patterns of metabolism and translocation in honeylocust seedlings were less distinct. Zimmermann (1964) reported that the translocation pattern in continuously growing shoots is somewhat complicated because movement from mature leaves is both upward into shoot tips and downward towards the roots. Thus phloem transport in internodes between mature leaves is bidirectional in nature. In aging

cottonwood leaves there is a shifting of the translocation pattern from upward to bidirectional and finally to a predominantly downward direction (Larson and Gordon, 1969). Relatively high rates of metabolic activity continued in all parts of honeylocust seedlings as leaf abscission began. The leaves of walnut seedlings labeled early in the growing season showed reduced levels of radioactivity in the fall as leaf abscission began. In these plants the roots accounted for 65 to 70 per cent of the total radioactivity. It appears that walnut redistributes many metabolities from the leaves as leaf abscission begins. Less redistribution of ^{14}C was observed in honeylocust seedlings as the growing season ended. High levels of ^{14}C remained in honeylocust leaves on September 21 as leaf abscission began.

In developing black walnut seedlings sucrose accounted for most of the radioactivity recovered when specific sugars were studied on July 27, a period of high photosynthetic activity. On September 7, a period of low photosynthetic activity, increased amounts of radioactivity were found in glucose, fructose, raffinose, and stachyose.

Light saturation points for photosynthesis determined in this study were based on very limited data. These results need to be verified and refined. There was substantial variation in rates of photosynthesis and respiration between individual trees of both species. A closer examination of the genetic variation in photosynthetic and respiratory rates is of great interest to genetcists as a

basis for tree improvement. There was also considerable variation in rates of photorespiration between individuals of each species that deserves further study.

The incorporation of $^{14}CO_2$ into ethanol-insoluble cellular components by black walnut and honeylocust was much more rapid than that previously found in conifers. These results may be useful in comparing photosynthetic efficiency between these two broad groups. Short term labeling studies would be of value in this area. Except for the limited examination of sugars, the present study did not attempt to study the seasonal incorporation of $^{14}CO_2$ into specific metabolities. Further study in this area is essential before the deciduous growth habit can be completely understood and effectively manipulated by man.

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APPENDIX

TABLE 10.--Variation in net photosynthesis between individual seedlings of black walnut and honeylocust on four dates during the first growing season.

Observation Number	Black Walnut											
	June 15			July 27			August 17			September 7		
	1,800 ft-c	3,000 ft-c	10,000 ft-c	1,800 ft-c	3,000 ft-c	10,000 ft-c	1,800 ft-c	3,000 ft-c	10,000 ft-c	1,800 ft-c	3,000 ft-c	10,000 ft-c
	mg CO_2 dm^{-2} hr^{-1}											
1.	3.7	4.7	2.2	3.0	7.0	10.4	3.5	4.4	4.4	1.4	1.0	1.3
2.	7.4	5.6	4.1	3.9	5.6	1.9	6.4	10.6	10.5	0.6	1.9	1.1
3.	3.8	4.5	2.8	8.1	9.2	7.9	3.8	5.0	5.4	1.4	1.4	0.7
4.	3.5	5.7	5.7	8.6	14.2	14.0	4.6	5.3	5.2	2.4	2.8	1.8
5.	3.7	4.4	5.5	5.7	8.0	5.0	7.0	8.7	9.8	1.2	0.8	1.4
Mean	4.4	5.0	4.0	5.9	8.8	7.8	5.0	6.8	7.1	1.4	1.6	1.3
	Honeylocust											
	July 13			August 10			September 1			September 21		
	4,000 ft-c	7,000 ft-c	10,000 ft-c	4,000 ft-c	7,000 ft-c	10,000 ft-c	4,000 ft-c	7,000 ft-c	10,000 ft-c	4,000 ft-c	7,000 ft-c	10,000 ft-c
	mg CO_2 dm^{-2} hr^{-1}											
1.	8.7	10.4	10.2	11.6	19.6	19.6	12.7	15.4	16.0	15.3	15.2	16.3
2.	10.8	13.2	10.8	13.9	16.0	16.0	6.8	11.2	12.9	11.3	10.2	7.0
3.	9.9	11.0	11.6	14.8	14.8	13.0	17.3	18.7	17.1	7.0	12.3	12.3
4.	11.4	13.3	8.3	9.9	14.6	12.7	14.6	15.8	15.7	14.0	17.3	13.8
5.	13.6	14.4	12.1	21.5	17.8	16.3	19.2	23.0	17.5	4.2	11.9	11.8
Mean	10.9	12.4	10.6	14.3	16.6	15.5	14.1	16.8	15.8	10.4	13.4	12.2

TABLE 11.--Variation in dark respiration between individual seedlings of black walnut and honeylocust on four dates in the first growing season.

Observation Number	Black Walnut			
	June 15	July 27	August 17	September 7
	----------mg CO_2 dm^{-2} hr^{-1}----------			
1.	6.2	4.2	7.6	9.2
2.	4.8	5.6	2.7	6.0
3.	5.8	8.1	5.0	5.7
4.	8.2	3.6	6.9	6.6
5.	6.6	3.3	5.8	10.1
Mean	6.3	6.2	5.6	7.6
	Honeylocust			
	July 13	August 10	September 1	September 21
1.	12.9	6.4	12.8	4.6
2.	8.4	6.0	4.9	[1]
3.	9.4	4.6	6.5	12.6
4.	7.6	10.0	7.7	3.7
5.	8.6	10.7	8.6	2.0
Mean	9.4	7.5	8.1	5.7

[1]Insufficient Data

TABLE 12.--Variation in photorespiration between individual seedlings of black walnut and honeylocust on four dates during the first growing season.

Observation Number	Black Walnut							
	June 15		July 27		August 17		September 7	
	3,000 ft-c	10,000 ft-c	3,000 ft-c	10,000 ft-c	3,000 ft-c	10,000 ft-c	3,000 ft-c	10,000 ft-c
	mg CO_2 dm^{-2} hr^{-1}							
1	2.0	6.7	6.4	[1]	6.9	7.5	3.2	[1]
2	5.7	7.6	6.8	8.7	7.2	5.7	5.4	4.9
3	7.1	6.4	6.7	13.0	7.4	7.7	4.5	11.5
4	12.4	11.2	8.3	16.7	6.9	9.3	2.8	6.4
5	4.9	10.2	9.8	7.4	10.9	6.1	[1]	[1]
Mean	6.4	8.4	7.6	9.2	7.9	7.3	4.0	7.6

	Honeylocust							
	July 13		August 10		September 1		September 21	
	7,000 ft-c	10,000 ft-c	7,000 ft-c	10,000 ft-c	7,000 ft-c	10,000 ft-c	7,000 ft-c	10,000 ft-c
	mg CO_2 dm^{-2} hr^{-1}							
1	19.0	1.6	8.8	[1]	30.1	15.4	9.8	[1]
2	3.0	1.2	6.7	3.8	12.0	20.9	[1]	0.0
3	7.8	2.3	3.4	4.7	22.5	26.0	5.2	1.8
4	7.0	9.8	15.9	6.5	13.6	16.3	3.1	0.6
5	7.2	10.3	15.8	27.1	22.1	29.0	0.6	0.0
Mean	8.8	4.8	10.1	8.4	20.1	21.5	4.7	0.6

[1]Insufficient data

TABLE 13.--Variation in the CO_2 compensation point between individual seedlings of black walnut and honeylocust seedlings on four dates during the first growing season.

Observation Number	Black Walnut			
	June 15	July 27	August 17	September 7
	ppm			
1.	160	110	[1]	235
2.	140	[1]	100	235
3.	130	130	165	[1]
4.	195	95	170	225
5.	175	135	145	[1]
Mean	160	118	145	232

Observation Number	Honeylocust			
	July 13	August 10	September 1	September 21[2]
	ppm			
1.	210	100	180	
2.	140	110	[1]	
3.	145	105	160	
4.	130	135	140	
5.	150	120	145	
Mean	155	114	156	

[1]Insufficient Data

[2]Data not obtained because of low rate of net photosynthesis

TABLE 14.--Seasonal effect on incorporation of ^{14}C into major fractions extracted from leaves, stem, and roots of black walnut seedlings after one day and at the end of the first growing season. Three seedlings were labeled on each of four dates during the growing season.

Percent of Total Activity in:	Ethanol Extract--Water Soluble									Ethanol Extract--Non-Water Soluble			Ethanol Insoluble			All Fractions Combined		
Plants Harvested	Amino Acids			Organic Acids			Sugars											
	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
After 24 Hours, Labeled on:																		
June 15	3.1	0.2	0.1	4.7	0.9	1.2	10.0	0.6	2.8	46.7	2.0	0.8	14.9	10.7	1.4	79.3	14.4	6.3
July 27	0.7	0.5	0.9	6.6	3.4	3.5	2.6	5.0	9.3	12.7	0.4	1.5	20.8	13.7	18.4	43.4	23.0	33.6
August 17	0.1	0.2	0.4	15.2	0.4	5.0	0.6	0.2	6.7	28.7	1.2	0.9	11.2	3.0	26.2	55.8	5.0	39.2
September 7	0.7	T	0.1	8.8	1.2	0.2	5.4	0.4	1.3	4.0	0.1	T	5.2	2.3	70.4	24.0	4.0	72.0
At end of Growing Season, Labeled on:																		
June 15	0.6	3.9	0.9	0.9	0.6	0.8	0.6	0.9	0.7	1.2	2.0	3.8	11.2	8.6	63.3	14.5	16.0	69.5
July 27	0.5	1.6	2.2	0.7	2.2	0.2	1.2	0.7	2.8	10.4	2.3	0.6	8.5	5.4	60.9	21.3	12.2	66.5
August 17	0.2	0.2	0.7	0.8	1.1	7.6	0.3	0.7	2.6	9.6	0.5	3.2	7.4	6.0	59.1	18.3	8.5	73.2
September 7	0.4	0.2	1.2	4.9	0.6	3.1	2.1	0.8	7.4	12.2	0.2	0.8	6.8	8.9	50.4	26.4	10.7	62.9

TABLE 15.--Seasonal effect on incorporation of ^{14}C into major fractions extracted from leaves, stem, and roots of honeylocust seedlings after 1 day and at the end of the first growing season. Three seedlings were labeled on each of four dates during the growing season.

Percent of Total Activity in:	Ethanol Extract--Water Soluble									Ethanol Extract--Non-Water Soluble			Ethanol Insoluble			All Fractions Combined		
	Amino Acids			Organic Acids			Sugars											
Plants Harvested	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
After 24 Hours, Labeled on:																		
July 13	3.0	1.8	0.4	6.5	0.9	0.7	2.2	0.4	0.9	5.8	4.4	6.8	32.4	12.0	21.8	49.9	19.5	30.6
August 10	3.7	1.3	1.7	8.1	3.8	3.8	1.8	4.6	5.2	39.2	4.0	1.9	7.6	7.0	6.3	60.4	20.7	18.9
September 1	0.6	0.5	0.3	1.3	0.6	0.4	9.9	26.2	2.6	21.2	7.1	1.8	12.9	7.0	7.6	45.9	41.4	12.7
September 21	0.1	0.4	0.2	1.5	0.3	2.0	0.6	1.3	3.9	5.7	0.6	1.9	3.0	26.4	52.1	10.9	29.0	60.1
At End of Growing Season, Labeled on:																		
July 13	1.8	3.5	0.7	2.5	0.3	1.1	1.8	0.8	2.1	7.0	10.9	4.3	10.7	12.8	39.9	23.6	28.3	48.1
August 10	0.4	0.2	0.1	1.9	0.4	1.7	0.4	0.4	0.4	2.9	5.3	2.5	38.6	23.7	21.1	44.2	30.0	25.8
September 1	0.3	0.2	0.4	2.2	0.4	3.2	1.4	1.4	0.5	7.0	3.6	3.3	32.0	16.9	27.2	42.9	22.5	34.6
September 21	0.4	0.8	0.3	2.1	1.3	0.9	0.4	3.0	9.8	28.3	4.1	2.5	3.2	18.7	24.2	34.4	27.9	37.7