OBSERVATIONS ON THE GROWTH AND PHYSIOLOGY OF PINUS STROBUS L. SEEDLINGS GROWN UNDER VARIOUS CONDITIONS OF SOIL MOISTURE AND NITROGEN AND PHOSPHORUS NUTRITION

by

G. R. Lister

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EXAMINING COMMITTEE APPROVAL

. . . . . .C. D. Nelson
Senior Supervisor

. . . . . .D. R. M. Scott
External Examiner

. . . . . .F. J. F. Fisher
Supervising committee

. . . . . .W. Vidaver
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FRONTISPIECE

A typical specimen of Pinus strobus L. used in these studies. This seedling is three and a half years old and was grown under intermediate nitrogen and phosphorus nutritional levels in an open cold-frame.


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PROLOGUE

A comprehensive study of tree physiology was initiated at Queen's University in 1958. This has lead to the publication of several papers by G. Krotkov, C. D. Nelson, V. Slankis, V. Runeckles, T. Shiroya and G. R. Lister.

Part of the continuing work has been written up as two publications[1] for the Annals of Botany and form the major portion of this thesis. In addition a brief Introduction has been added to place the papers in perspective.

[1] Lister, G. R., Slankis, V., Krotkov, G. and Nelson, C. D., 1967. Physiology of Pinus strobus L. seedlings grown under high or low soil moisture conditions. Ann. Bot. N.S. Vol. 31, No. 121, 121 - 132.

Lister, G. R., Slankis, V., Krotkov, G. and Nelson, C. D., 1967. The growth and physiology of Pinus strobus L. seedlings as affected by various levels of nitrogen and phosphorus. Ann. Bot. N.S. (In Press, accepted).

ABSTRACT

PART I

White pine seedlings (Pinus strobus L.) were grown at high or low soil-moisture levels. The leader stem length, fresh weight of the seedlings, respiration, photosynthesis, transpiration, translocation of photosynthate from shoots to roots, and bio-electric potentials between the tip and the base of the stem were measured throughout the growing season from April to October.

At both moisture levels the lowest translocation of recent photosynthate from shoots to roots occurred during early summer, or at the time when the rate of root growth was the lowest and that of the shoot the highest. During early summer the specific activity of $^{14}CO_2$ respired by the shoots of such plants remained high throughout an 8 h experimental period, indicating a continuous utilization of recent photosynthate as a respiratory substrate. On the other hand, early and late in the growing season, when translocation of recent photosynthate from shoots to roots and the rate of root growth were high, the specific activity of $^{14}CO_2$ respired by the shoots rapidly decreased during the 8 h experimental period, indicating a drop in the utilization of recent photosynthate as respiratory substrate. The highest positive values for the potential difference between the top and the base of the main shoot also occurred in early summer or during the period of high rates of transpiration.

PART II

Potted white pine (Pinus strobus L.) seedlings were grown on media containing different amounts of nitrogen (N) and phosphorus (P). The seedlings were grown either in controlled environment chambers or outdoor cold frames. Following periods of five, seven and thirteen weeks on treatment the seedlings were analyzed to determine rates of respiration, photosynthesis and the degree of translocation of recent photosynthate to the roots. Shoot and root fresh weights were recorded. Analyses were made to determine the metabolic fate of the translocated sugars.

The best overall growth and the highest root/shoot ratios were found in seedlings receiving intermediate levels of N and P. The range of nutritional conditions employed was found to have no effect upon rates of shoot and root respiration or photosynthesis, even after thirteen weeks of treatment.

Lateral root formation was depressed under conditions of high N and P. Mycorrhizal abundance showed a maximum at intermediate levels of nutrients. Translocation of recent photosynthate to the roots was depressed by high P, this depression was however, reversed to some extent by increasing N levels.

The hydrolysis of sucrose recently translocated to the roots was increased with increasing N supply. The resultant hexoses being metabolised to amino and organic acids. However, sucrose continued to be the dominant form in which

recently translocated ^{14}C occurred in the roots. High levels of P reduced the effect of N on the metabolism of translocated sucrose.

Relatively very few of the metabolically active compounds, isolated from the soluble fraction of the roots, showed distinct patterns of change which could be correlated to either the different nutritional levels of N and P supplied or to the incidence of mycorrhizae.

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GENERAL INTRODUCTION

Kramer and Kozlowski in the introduction to their book 'Physiology of Forest Trees' (McGraw-Hill, 1960), wrote the following of trees and the plant physiologists who study them.

"A tree means different things to different people. For our ancestors it was the chief source of fuel and shelter and occasionally an object of worship. To the average man on the street it may be a source of pleasant shade in the summer but a nuisance which sheds leaves on his lawn in the autumn....to a forester (they are) a source of timber and pulpwood. To a physiologist, however, a tree is a complex biochemical factory which starts from a seed and literally builds itself, and physiologists are....interested in the manner in which the complex of processes that we call 'growth' is carried on. The success of trees depends primarily on their efficiency in manufacturing carbohydrates....and their ability to convert these simple carbohydrates into new tissues. This involves translocation of the products of photosynthesis to various parts of the plant....and their use in assimilation and respiration....(While) Plant physiologists are interested primarily in learning how trees grow....foresters and horticulturists are interested primarily in how to grow trees more efficiently. These two approaches are more closely related than many people suppose....Each type actually contributes to the other, and the greatest....progress will occur when physiologists learn more about the growing of trees, foresters....learn more about the physiology of trees, and the two work together to solve the many problems which exist."

The amount of basic research in Plant Physiology in which trees served as the experimental material was small until relatively recent times. Consequently the present concepts of the physiology of trees is based in large part upon analogy, by transfer of information obtained from other plant species supported by relatively few direct experiments.

There is no evidence to suggest that trees are in any way basically different from other plants.

They do, however, present peculiar problems because of their particular gross form, e.g. long distance transport and integration, extreme perennial nature, e.g. site conservation and ability to survive adverse or fluctuating environments outside of a specialized state (seeds or spores), and the length of the life cycle, e.g. genetics and breeding experiments. Biochemically trees produce unique arrays of metabolites, e.g. lignins, resins etc., that have proven somewhat intractable to analytical procedures both from the point of view of clarifying their pathways of metabolism and also by their interference in studies of the general metabolism.

Basic work on trees has been, and still is in two general areas. First, the seedling stage - 0 to 5 years, and secondly, mature trees - 20+ years. Study programmes have been set up to follow the progress of trees from seed to maturity. These programmes generally are concerned with growth, morphology, dry matter production, and provenance studies. Such long-term projects have their obvious limitations. The initiating worker rarely is on site more than 10 to 15 years; in fact the programme may outlive him. Progress in methodology, together with new knowledge, may lead to the partial or full abandonment of many programmes before the projected time span elapses.

The sheer size of mature trees presents considerable difficulties to the physiologist, 80 to 90% of whose time may be spent in solving problems arising from the scaling up of laboratory or seedling type experiments to natural stand conditions. The number of such studies that can be done is

limited, as are the replications and site differences.

For the above reasons most of the physiological studies on trees have been carried out on seedlings or cut branches brought into the laboratory. The latter type has in many instances the severe limitation of a lack of information as to the extent to which the data obtained are affected by the isolation of the organ from the whole organism. Work on seedlings has been favoured because of the relative ease with which they can be handled in the laboratory and also because it is the first few years that are very critical from the survival point of view. Competition from other individuals and species is intense and the ability to withstand adverse environmental conditions is limited.

Failure of many afforestation projects carried out between 1910 and 1935 on prairie type soils, for example, in Wyoming and Australia (Hatch 1936 and 1937), emphasized the importance of ectotrophic mycorrhizal systems to the survival of trees under low soil fertility conditions. These failures stimulated further work on the mycorrhizal phenomenon.

The two main theories that have been put forward to account for the observed distribution of mycorrhizal roots in nature are as follows. First, the Stahlian-Hatch theory which states that when the appropriate innoculum is present and other environmental factors are favourable, then the incidence of mycorrhizae and their development varies inversely with the availability of mineral nutrients in the substrate (Stahl,

1900; Hatch,1937). Second, Bjorkman (1942) proposed a 'carbohydrate' theory.

"The mycorrhiza fungi parasitize the roots for soluble carbohydrates. They do not enter the roots unless these contain a certain amount of soluble carbohydrates, that is, only if and when photosynthesis is rapid enough and is not followed on the heels by the synthesis of proteids. Now with plenty of nutrients the plant is able to build up proteids as fast as the photosynthetical output of carbohydrates permits. There will be little for the fungi to seek in the roots, and no mycorrhiza will form. Only if there is a certain scarcity of one or other nutrient like nitrogen and phosphorus, needed for the higher syntheses, will these slow down and sugar pile up, inducing the fungi to enter the roots and form mycorrhiza. Too severe a scarcity of some essential nutrient may slow down the primary syntheses as well, causing a scarcity even of soluble carbohydrates and hence poorer conditions for mycorrhizal infection. Phosphorus, in particular, can be expected to have a double effect, since it is known highly to stimulate the production of carbohydrates and at the same time is needed as a building stone for the synthesis of proteids."

These two theories are not wholly incompatible, the main difference being that the emphasis as to the dominant factor(s) differs. However, both theories say little or nothing about the mechanism of initiation and establishment of the symbiotic relationship. For example, how is the apparent stimulus of a surplus of carbohydrate in the roots exerted upon the rhizosphere mycorrhizal fungi? Does the scarcity of one or more nutrients slow down carbohydrate metabolism in the root and with what consequences? Are there any changes in the general metabolism of the roots which correlate with and may account for the observed occurrence of mycorrhizae?

If carbohydrate metabolism is the primary factor involved in initiation and maintenance of the mycorrhizal association, then investigation should be made of the flow of carbon from

source (shoot) to sink (root) and of its subsequent fate there. In what form is the carbon first elaborated and in what form and what amounts is it moved from source to sink under various environmental conditions. Possession of such information would then facilitate the investigation of the metabolic fate of the carbon in the root under various conditions of mineral nutrition. Possible correlations between such data and the observed mycorrhizal abundance and the general growth of the tree may then be sought, in turn leading to some possible clarification of the mycorrhizal phenomenon. Such a programme was initiated several years ago at Queen's University, Kingston, Ontario.

Over many years a wealth of knowledge in the application of isotope techniques to plant physiological studies has been built up in the Queen's Laboratory under Dr. G. Krotkov. The development of an Infra-red Gas Analyzer system, Lister et al (1961) (Figures A_1, A_2, and A_3) allowed the coupling of these two techniques and their subsequent application to problems in tree physiology. This work was carried out in conjunction with the Forest Pathology Laboratory at Maple, Ontario, where in co-operation with Dr. V. Slankis and co-workers we have resolved many of the problems involved in the successful growth of pine seedlings under partially and fully controlled environmental conditions. Figures B_1, B_2, C_1 and C_2 show the construction and layout of the special cold-frame described in Part I and II for the growth of seedlings under partially controlled environmental conditions. Figure D shows typical examples of

white pine seedlings grown in the open cold-frame. Figure E illustrates the manner in which the bio-potential data (Part I) was obtained.

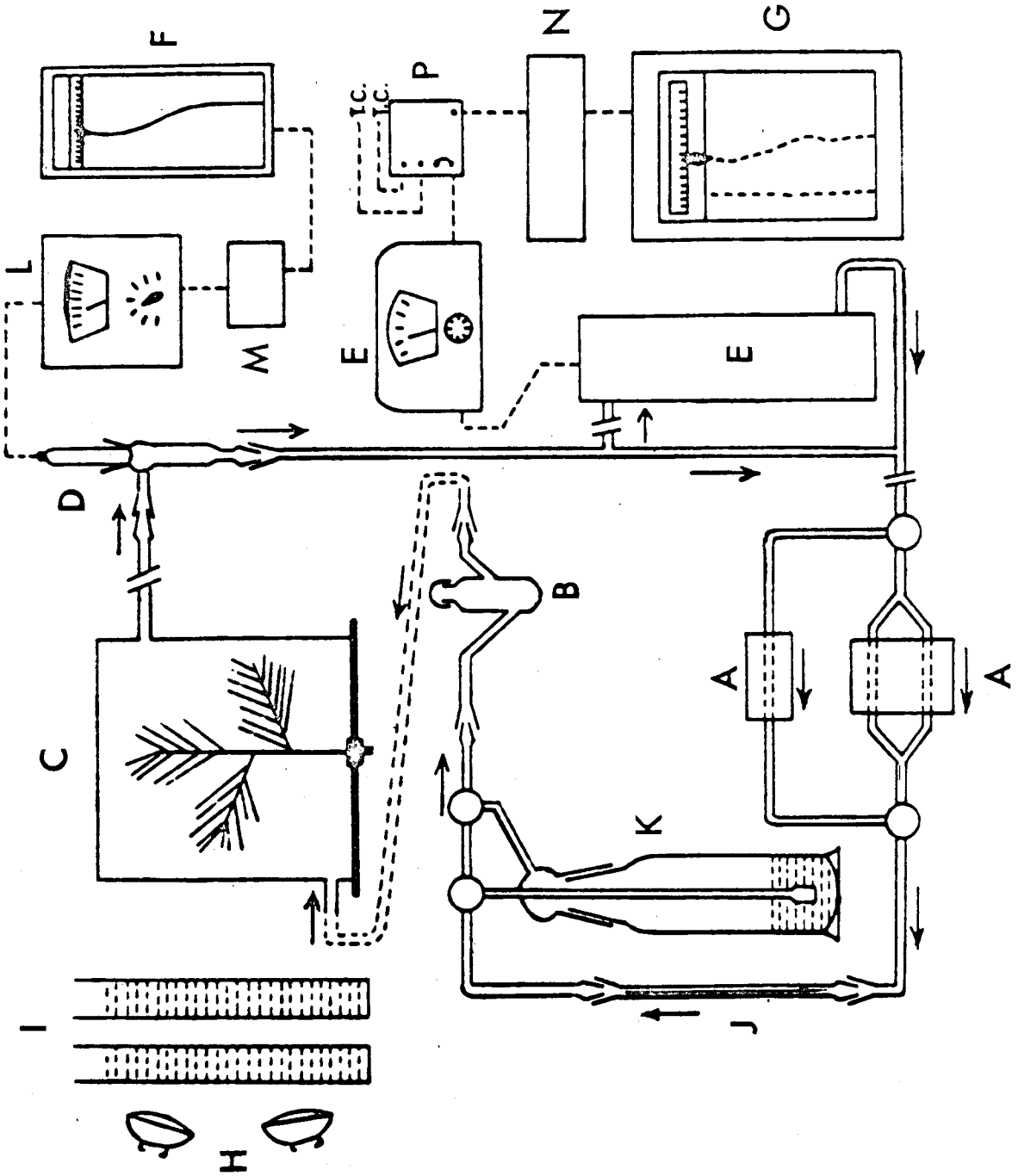
The early work of Shiroya and Slankis et al (1962) and Shiroya and Lister et al (1962) provided valuable background data on the physiology of P. resinosa and P. strobus seedlings. The nature of the photosynthetic products and the form in which the elaborated carbon is translocated was elucidated. Their data also showed that the translocation of assimulated carbon to the roots was markedly affected by the light intensity under which the plants were grown. Since the length of day as well as the light intensity varies with season, one might therefore expect seasonal changes in the translocation of photosynthate to roots. Such proved to be the case as was demonstrated by Shiroya et al (1966). They found that not only did the supply of carbon to the roots vary with season, but that the root growth and mycorrhizal abundance (Slankis, unpublished data) paralleled the changes.

The experiments reported in this thesis are a continuation of the above programme. In Part I, the seasonal changes in degree of translocation of carbon to the roots of pine seedlings is further investigated particularly with respect to soil moisture regime during growth. Part II reports on an investigation into the fate of the translocated carbon in the roots of seedlings grown under various levels of nitrogen and phosphorus nutrition and the effects of the various nutrient levels on

the general physiology of the seedlings and on the incidence of mycorrhizae.

Figure A_1 - Diagram of Closed-Circuit Apparatus

A - Pumps
B - $^{14}CO_2$ generator flask
C - Plexiglass plant chamber
D - Geiger-Muller tube
E - Infra-Red Gas Analyzer
F - Recorder
G - Multichannel recorder
H - Lamps
I - Heat filters (20cm water)
J - Flowmeter
K - Alkali CO_2 absorption tower
L - Radiation Monitor
M - Amplifier
N - Pre-amplifier
P - Solanoid step switch
TC - Thermocouples. (Plant chamber temperature, root temperature and Relative Humidity of the air stream.)


F
N
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P
T.C.
T.C.
L
M
E
E
D
B
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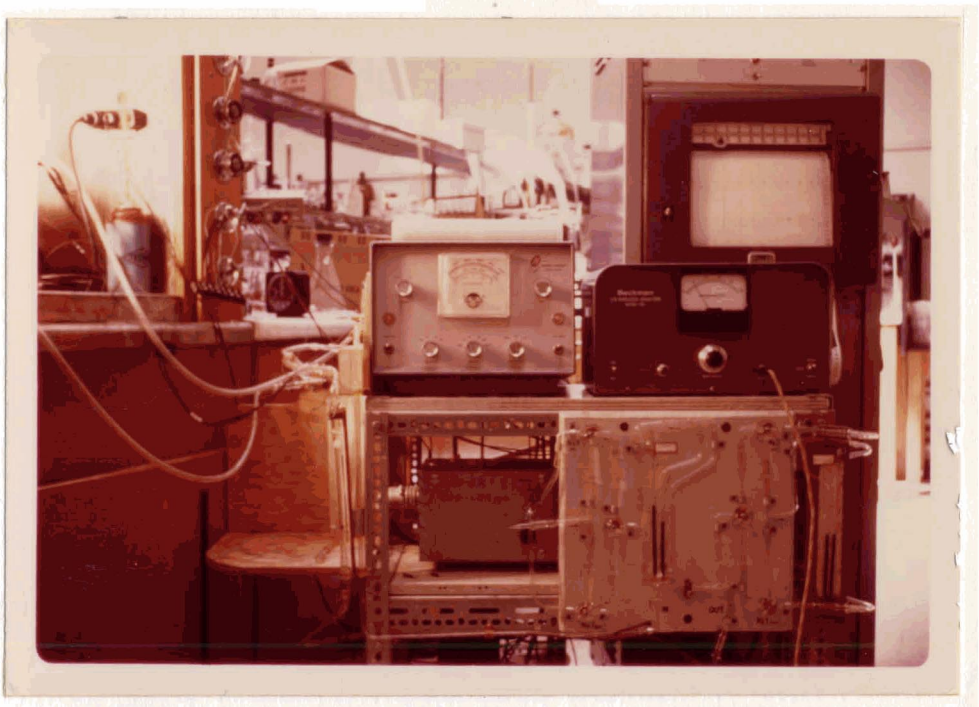
C
K
I
J
H

Figure A_2.

Closed-circuit Infra-Red Carbon Dioxide Analyser and $^{14}CO_2$ monitoring system.

Figure A_3.

Seedling during $^{14}CO_2$ feeding period.



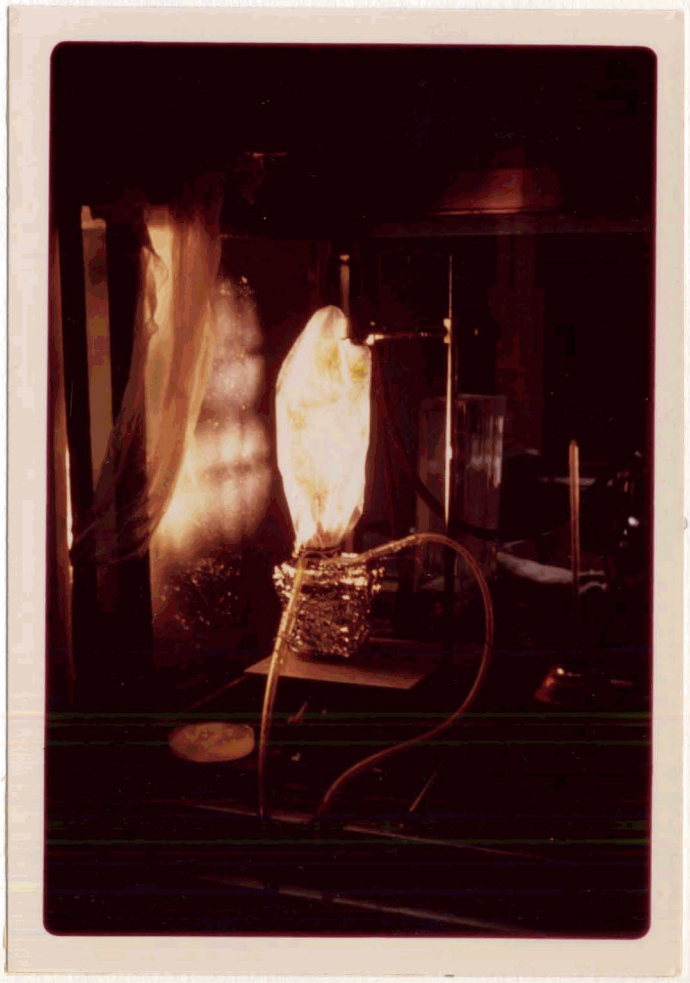


Figure B_1.

Open cold-frame, Forestry Pathology Laboratory, Maple, Ontario.

Figure B_2.

Arrangement of seedlings in open cold-frame.

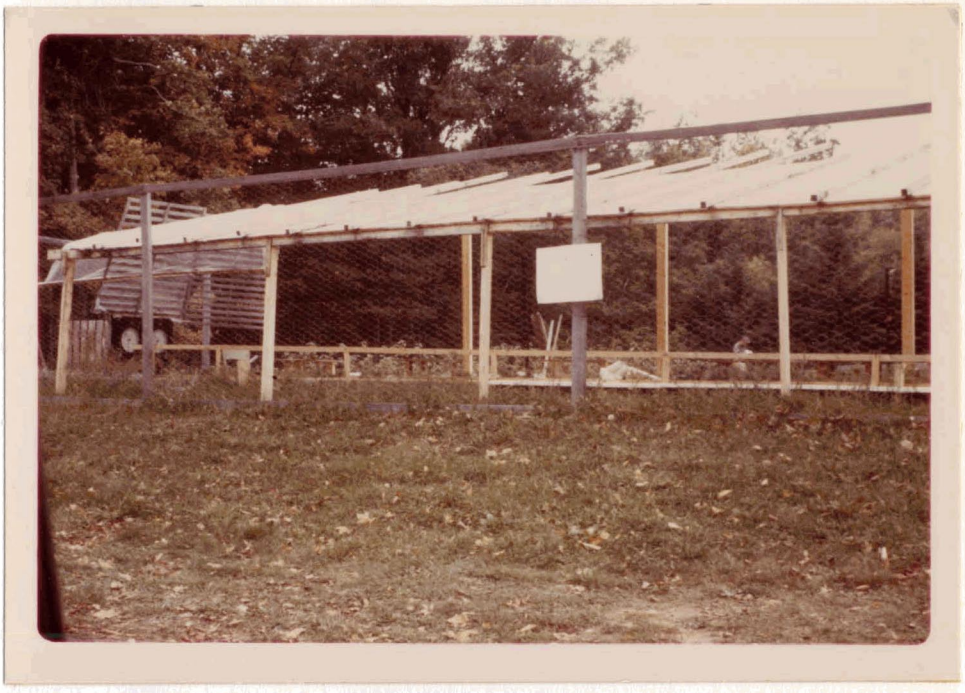


Figure C_1.

Seedlings in cold-frame with protective (temperature and precipitation) styrofoam strips in place.

Figure C_2.

Seedlings in cold-frame with styrofoam protection strips removed. Cavity beneath is temperature controlled to maintain a root temperature of 18-19°C and an R.H. of 85-90%.


35


40

Figure D.

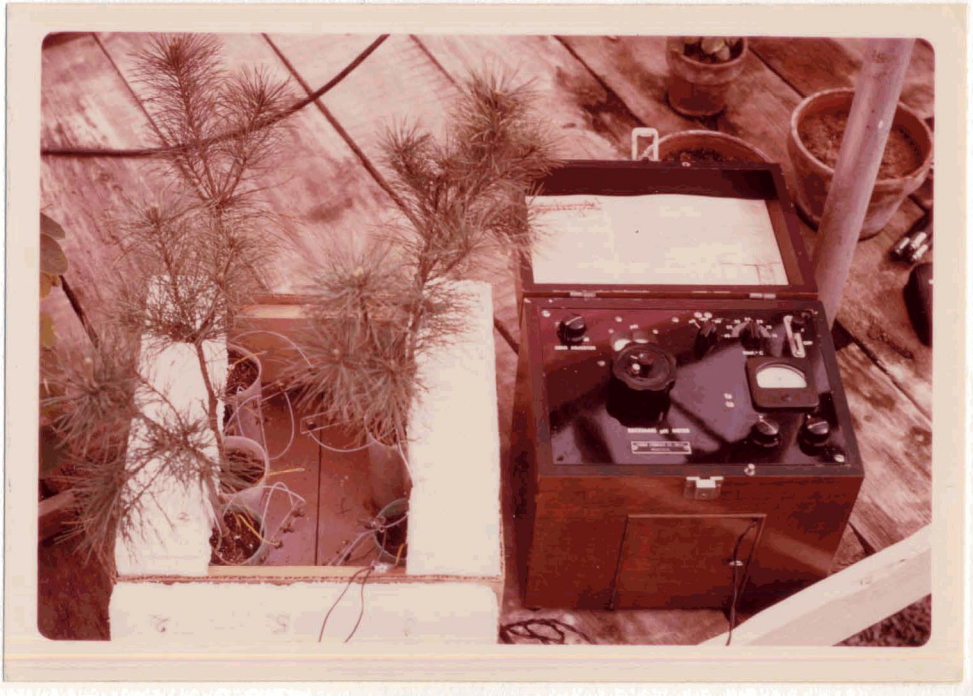
Typical examples of high (HM) and low (LM) soil moisture grown seedlings five months after commencement of treatment in the open cold-frame.

Note the difference in the extent of new needle development.

Figure E.

Measurement of Bio-potentials. (Beckman Model G pH meter used as a portable 0-1400 mV potentiometer)


1,458 gm
28
53
8-64-I_{LM}
8-64-I_{HM}



P A R T I

Physiology of *Pinus strobus L.* Seedlings Grown under High or Low Soil Moisture Conditions.

INTRODUCTION

In the course of our investigations into the physiology of conifers, we have observed a seasonal pattern of translocation of recent photosynthate from shoots to roots of white pine seedlings (Shiroya et al., 1966). For a short period in the spring, and for a longer period in the late summer and autumn, translocation of recent photosynthate from shoots to roots is relatively high. For a short period in the late spring or early summer, and during the winter, translocation from shoots to roots is low or non-existent.

Kursanov (1961) suggested that the level of root metabolic activity affects the degree of the 'pull' of assimilates to the roots. If, therefore, the reduced root growth in the late spring or early summer is a result of the low soil-moisture conditions characteristic of this time of the season, as has been suggested by Turner (1936) and Reed (1939), then growing plants under low soil-moisture conditions throughout the growing season should result in continuously low levels of translocation to their roots. On the other hand, plants grown under constant optimal or near optimal levels of soil moisture might be expected to show continuous root growth and translocation of photosynthate from shoots to roots throughout the whole season.

There have been several reports in the literature on the bio-electric phenomena in trees. Parr (1943) observed correlations between the stem potentials and susceptibility to insect attack. Wilner (1961) found a relationship between the electrical resistance of the wood and frost hardiness in various varieties of apple-trees. Fensom (1955 and 1963) observed seasonal changes in resistance, conductance, and potential differences in several tree species and found that seasonal patterns are influenced by temperature, rainfall, and by some factors inherent in the living tree itself, such as, for example, the growth-regulator levels. As Fensom (1963) wrote, '...an empirical study of electrical properties in such complex tissue as a living tree may be valuable to biologists, not only as a reservoir of information to sustain advancing theory, but also as was electro-cardiography, a phenomenon useful in its own right to throw light upon the internal functioning of the organism, once the patterns of normality have been established.'

The experiments reported below were carried out to ascertain: (a) whether the maintenance of constant moisture conditions in the soil at either high or low levels, would eliminate the seasonal periodicity of translocation from shoots to roots as observed normally; (b) whether the magnitude of translocation of photosynthate from shoots to roots was high in plants grown under high soil moisture, as compared with those grown under low soil moisture; and (c)

whether there was any correlation between the seasonal changes in the bio-electric potentials in the stem and any of the physiological characteristics measured.

MATERIALS AND METHODS

White pine seedlings, Pinus strobus L., were used as experimental material. The seedlings were grown for 2 years in open seedbeds at the Department of Lands and Forests Nursery, Midhurst, Ontario. In late April, immediately after the ground thawed, a number of these seedlings were transplanted into individual pots filled with the same sandy-loam soil taken from the original seedbeds. The pots were placed in a special cold-frame at the Laboratory of Forest Pathology, Maple, Ontario. The construction of this cold-frame was such that the pots were easily removed for the maintenance of their soil-moisture regime, while being covered, and therefore protected from rainfall, by strips of styrofoam sheet through which only the shoots protruded. A wide transparent polythene canopy was placed 8 ft overhead, protecting the plants from all but near horizontal storm-driven rain. Pot-soil and crown level air temperatures were continuously recorded and were found never to vary by more than 2° C from comparable measurements made in the adjacent open seedbeds.

The seedlings were divided into two groups: one was maintained under the conditions of high soil moisture at an average of $15 \cdot 3 \pm 3 \cdot 5$ per cent, and the other under low soil moisture at an average of $9 \cdot 3 \pm 2 \cdot 0$ per cent. The soil-moisture levels were maintained by weighing individual pots with seedlings every day and adding sufficient water to attain the predetermined weight of each potted seedling. This weight was adjusted every 7 to 10 days to correct for the increase in the seedling weight due to its growth. Several pots containing only soil were interspersed with the potted seedlings. The water loss from these pots gave the magnitude of evaporation from the pots themselves and permitted calculation of the water loss from a potted seedling via the seedling alone, i.e. transpiration.

Seven seedlings from each high or low moisture-level group were allocated for the determination of changes in their bio-electric potentials. These seedlings were interspersed in the cold-frame with the other experimental seedlings. Two silver-silver chloride wire electrodes were introduced into each seedling. One electrode was inserted through the base of the stem 3 to 5 mm above the emergence of the first lateral root and the other through the top of the same stem directly below the dormant apical bud. The distance between these two electrodes varied in different seedlings from 8 to 10 cm. The wires outside of each seed-

ling were insulated by placing plastic catheter tubing over them. The potential difference between the electrodes was measured every 2 to 3 days, prior to the daily soil-moisture adjustment, by means of a Beckmann pH meter, laboratory model type 'G'.

At approximately three-weekly intervals two or three seedlings from each group were brought to the laboratory at Queen's University. The shoot of each seedling was enclosed separately in a plastic chamber connected to a closed system containing an infra-red gas analyser, a Geiger tube and a pump. After a 1 1/2 h induction period in the light at 2500 f.c., a known amount of $^{14}CO_2$ was released into the system. The seedling was allowed to take this up to completion. At the end of 1 h the lights were turned off for a period of 15 min. during which time the rate and specific activity of the CO_2 respired by the shoot were determined. This rate represented the initial respiration rate of the shoot. The lights were then turned on again for a further 7-h period, with the seedling remaining in the closed system. At the end of a total of 8 h from the time of $^{14}CO_2$ release, the lights were again turned off and a second determination of the shoot respiratory rate was made. This rate represented the final respiration rate of the shoot. The seedling was then removed from the chamber and divided into its shoot, basal (outside of the photosynthesis chamber) part of the stem, and the roots. Following separation of roots from

the soil, the root respiration rate was also determined. The shoot, basal part of the stem, and roots were then weighed, killed, and extracted separately in boiling 80 per cent ethanol. Aliquots of the ethanol extracts were plated and counted to determine the distribution of ^{14}C throughout the seedling. Previous experiments have indicated that the ethanol-insoluble ^{14}C of such seedlings accounts for about 12 per cent of its total ^{14}C.

Throughout the experimental period of 8 h, temperatures of the shoots and roots were maintained at 25±2°C and 19±1°C respectively. The light source was two Silvania Photo Floods (type R.30, 3,400° K colour temperature) which provided an experimental light intensity of 2,500 ft-c (180 x 10^3 ergs/cm2/sec.) after Filtration through 20 cm. of water.

These experimental procedures and techniques were described in detail by Lister et al. (1961), Shiroya and Slankis et al. (1962) and Shiroya and Lister et al. (1966).

RESULTS

The results obtained for both High Moisture Grown (HMG) and Low Moisture Grown (LMG) seedlings are essentially similar and they are given in Figs. 1, 2, 3, and 5. Fig. 4 presents data on the air and soil temperature conditions under which seedlings were grown in the cold-frame.

As is seen in Fig. 1A, the leader-stem elongation precedes in time that of the new needles and is essentially completed by the time the new needles have attained about half of their final length. Stem elongation takes place over a period of 35-40 days as compared to the 85-95 day period of needle elongation. Although the growth period of the new shoot is essentially the same for both HMG and LMG seedlings, the rates of growth and the final length of both the leader stem and the new needles attained by the LMG seedlings are somewhat lower than those of the HMG seedlings.

The basal stem growth, Fig. 1B, measured in terms of both fresh weight and diameter, has two periods of relatively high rates: one in the spring and the other in the autumn, with a minimum during early or mid-summer.

The rate of apparent photosynthesis of the shoot, Fig. 1C, increases throughout the season, reaching a peak in late September or early October, after which the winter decline begins.

The rate of shoot respiration, Fig. 1D, rises to a maximum in early June and then declines steadily throughout the remainder of the growing season.

The total fresh weight of the root, Fig. 2A, follows essentially the same pattern as that of the basal stem, Fig. 1B. The root growth-rate has maxima in the spring and early autumn and a minimum in the early or mid-summer.

The fact that the highest rates of the root growth are at the beginning and at the end of the season, while that of the shoot is in the early summer, results in the root/shoot ratio, Fig. 2B, being high in the spring, decreasing or remaining constant during the summer, and then increasing again through the autumn.

The respiration rate of the roots, Fig. 2C, approximately parallels that of the root growth, Fig. 2A. It is highest during the periods of active root growth in the spring and early autumn and at a minimum in the mid-summer.

The pattern of translocation of recent photosynthate from shoots to roots, Fig. 2D, is similar to that of root growth and respiration. It is bimodal, having a small peak in the spring, an early summer minimum, and a large peak in the autumn.

Transpiration per seedling per week, Fig. 3A, increases steadily, reaching its first and main peak in mid-July and the secondary peak in early September. This pattern of transpiration rise and fall follows seasonal changes in the crown air and soil temperatures, Figs. 4A and 4B. On the other hand, variations in the rate of transpiration per unit needle surface area, Fig. 3B, follow only approximately the temperature data, the summer peak preceding the highest air temperatures in July and the September peak lagging behind the high air temperatures in late August.

The bio-electric potential difference between the top

and the base of the main stem, Fig. 5A, begins with low positive values early in the season. It goes up, reaching high positive values late in June or early in July. This is followed by a sharp decline to negative values, after which the potential difference alternates for the remainder of the season with much smaller amplitude of variation.

Fig. 5B shows the specific activity of the $^{14}CO_2$ respired by the shoot at the end of the experiment, or 7h after the uptake of $^{14}CO_2$, expressed as a percentage of that respired at the beginning or shortly after the uptake of $^{14}CO_2$. From the examination of this figure it is clear that this percentage is not constant during the season. It begins with a relatively low value in May, goes up in June and July, declines until the end of August, and then goes up again in September and October. This seasonal periodicity has a remarkably close inverse relationship to the translocation of recent photosynthate from shoots to roots, Fig. 3B.

Figure 1.

Seasonal changes in the length of the new shoot and needles (A), diameter and the fresh weight of the basal part of the stem (B), apparent photosynthesis (C) and respiration (D) of the shoot of P. strobus L. seedlings grown under either high (HMG) or low (LMG) soil moisture conditions. Each point is an average of two or three seedlings.

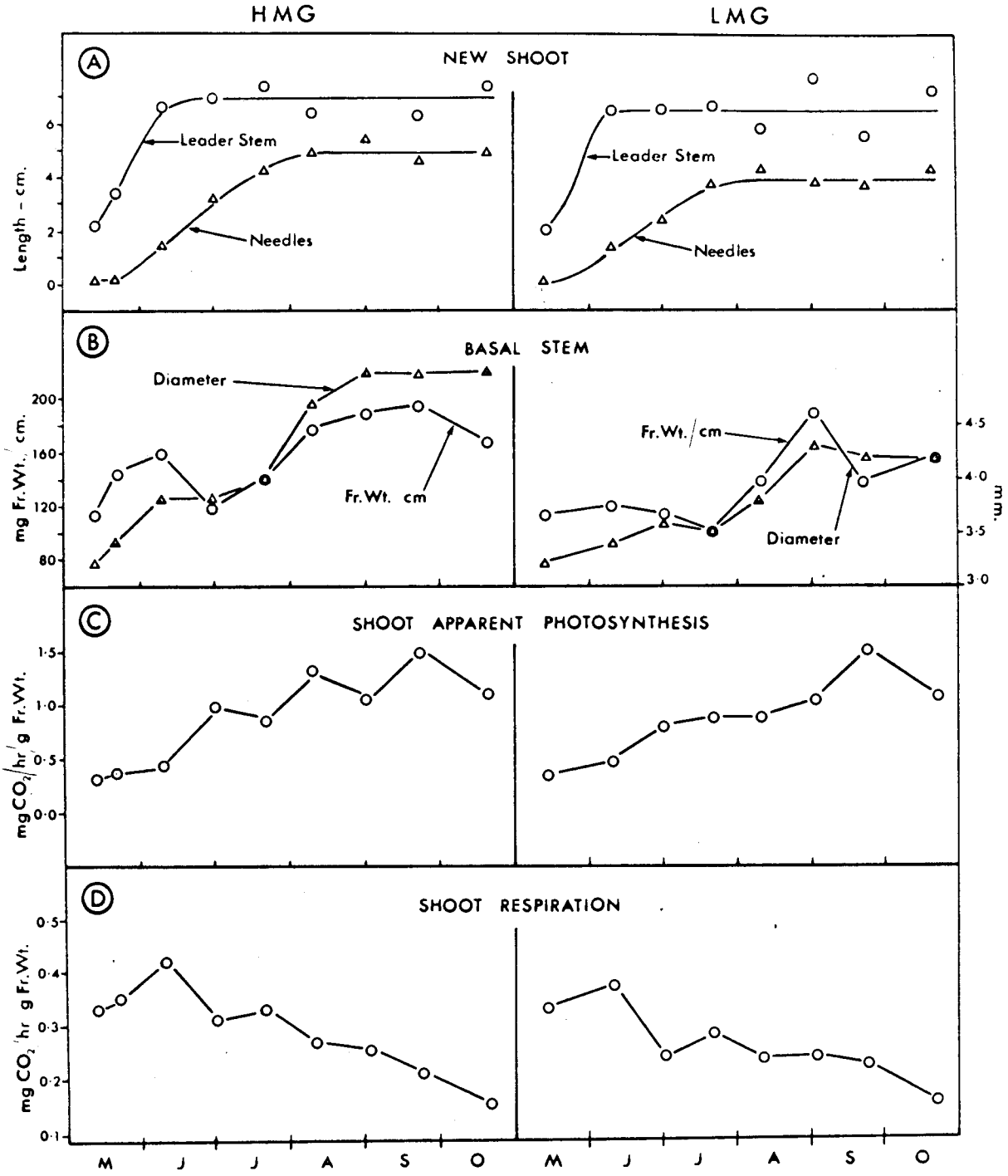

HMG
LMG
A
NEW SHOOT
Leader Stem
Needles
Length - cm.
B
BASAL STEM
Diameter
Fr.Wt. cm
mg Fr.Wt./ cm.
Fr.Wt./cm
mm.
C
SHOOT APPARENT PHOTOSYNTHESIS
mgCO2/hr/g Fr.Wt.
D
SHOOT RESPIRATION
mg CO2 /hr g Fr.Wt.
M J J A S O

Figure 2.

Seasonal changes in the total fresh weight of the root and the rate of root growth (A), root/shoot ratio (B), root respiration (C) and translocation of recent photosynthate from shoot to the root (D) of P. strobus L. seedlings grown under either high (HMG) or low (LMG) soil moisture conditions. D-^{14}C recovered in the root as percentage of that absorbed by the shoot. Each point is an average of two or three seedlings.

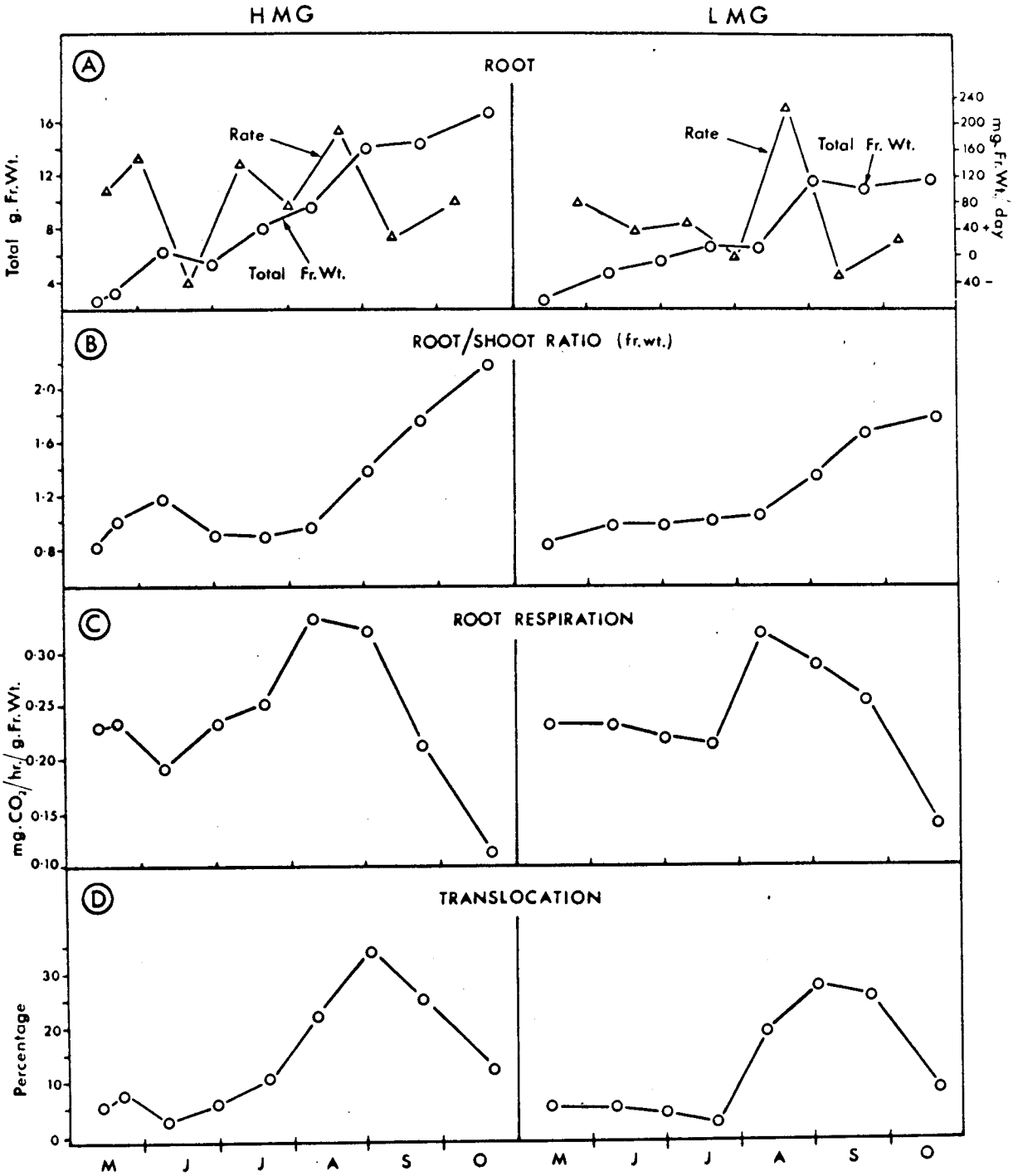

HMG
LMG
A
ROOT
Rate
Total Fr. Wt.
Total g. Fr. Wt.
mg. Fr. Wt./ day
B
ROOT/SHOOT RATIO (fr. wt.)
C
ROOT RESPIRATION
mg. CO2/hr./g. Fr. Wt.
D
TRANSLOCATION
Percentage
M
J
J
A
S
O

Figure 3.

Seasonal changes in the transpiration rate of P. strobus L. seedlings grown under either high (HMG) or low (LMG) soil moisture conditions. Expressed per seedling (A) or per dcm^2 of needle stomatal surface area (N.S.S.A.) (B). Each point in A is an average for twenty-five seedlings; in B an average for two or three seedlings.

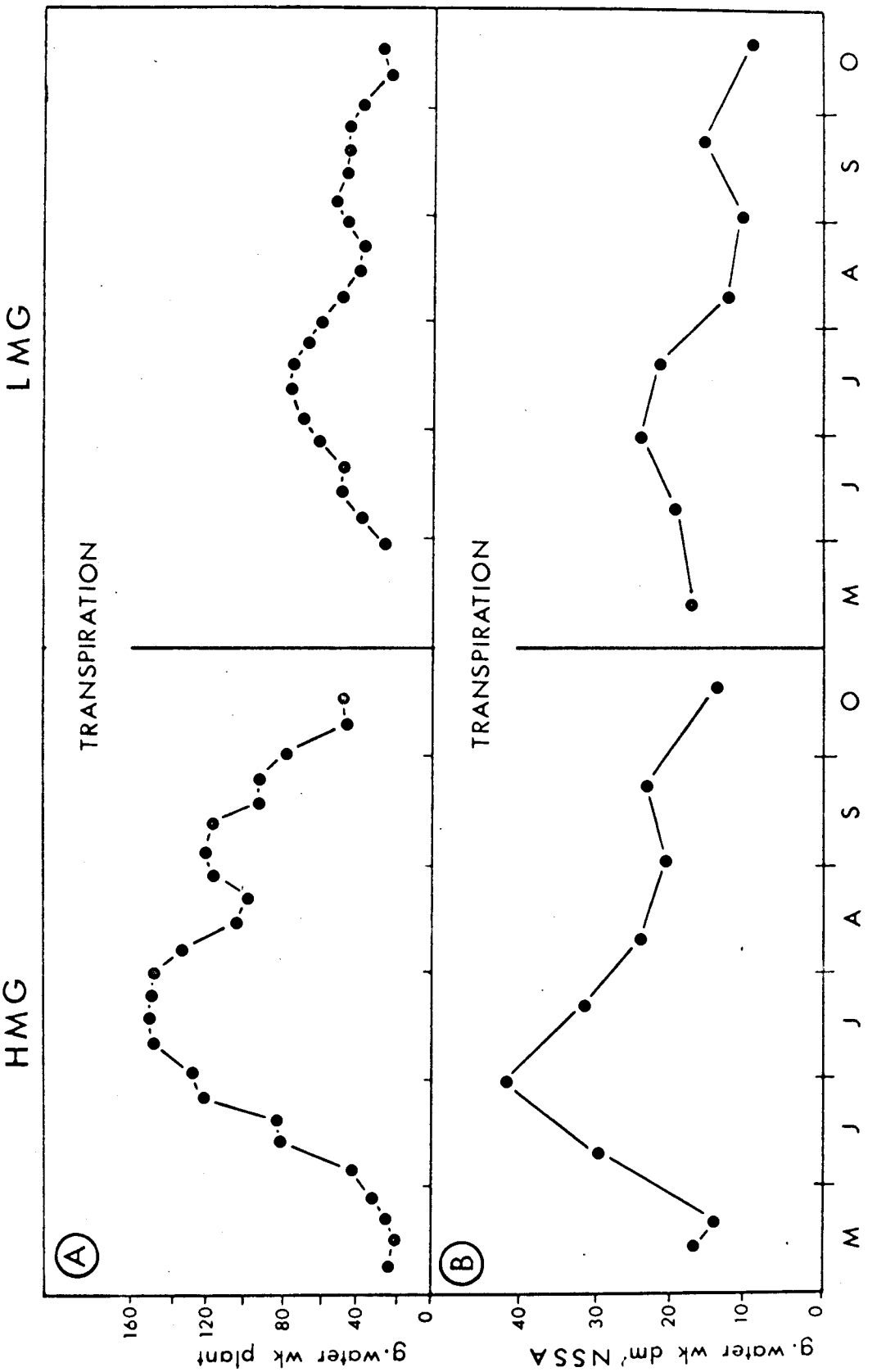

HMG
LMG
TRANSPIRATION
TRANSPIRATION
A
B
g.water wk plant
g.water wk dm² NSSA
0
40
80
120
160
0
10
20
30
40
M
J
J
A
S
O
M
J
J
A
S
O

Figure 4.

Seasonal changes in the temperature of the crown air (A) and pot soil (B) of P. strobus L. seedlings grown under high (HMG) or low (LMG) soil moisture conditions. B - Sensing element 10 cm. below soil surface.

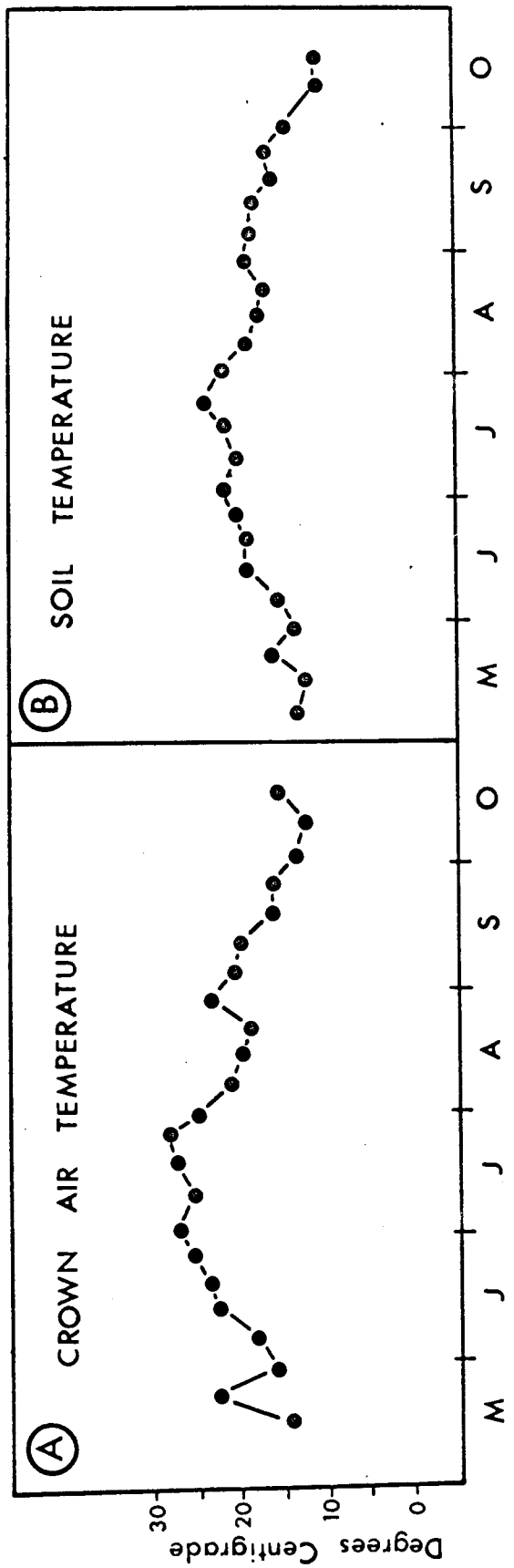
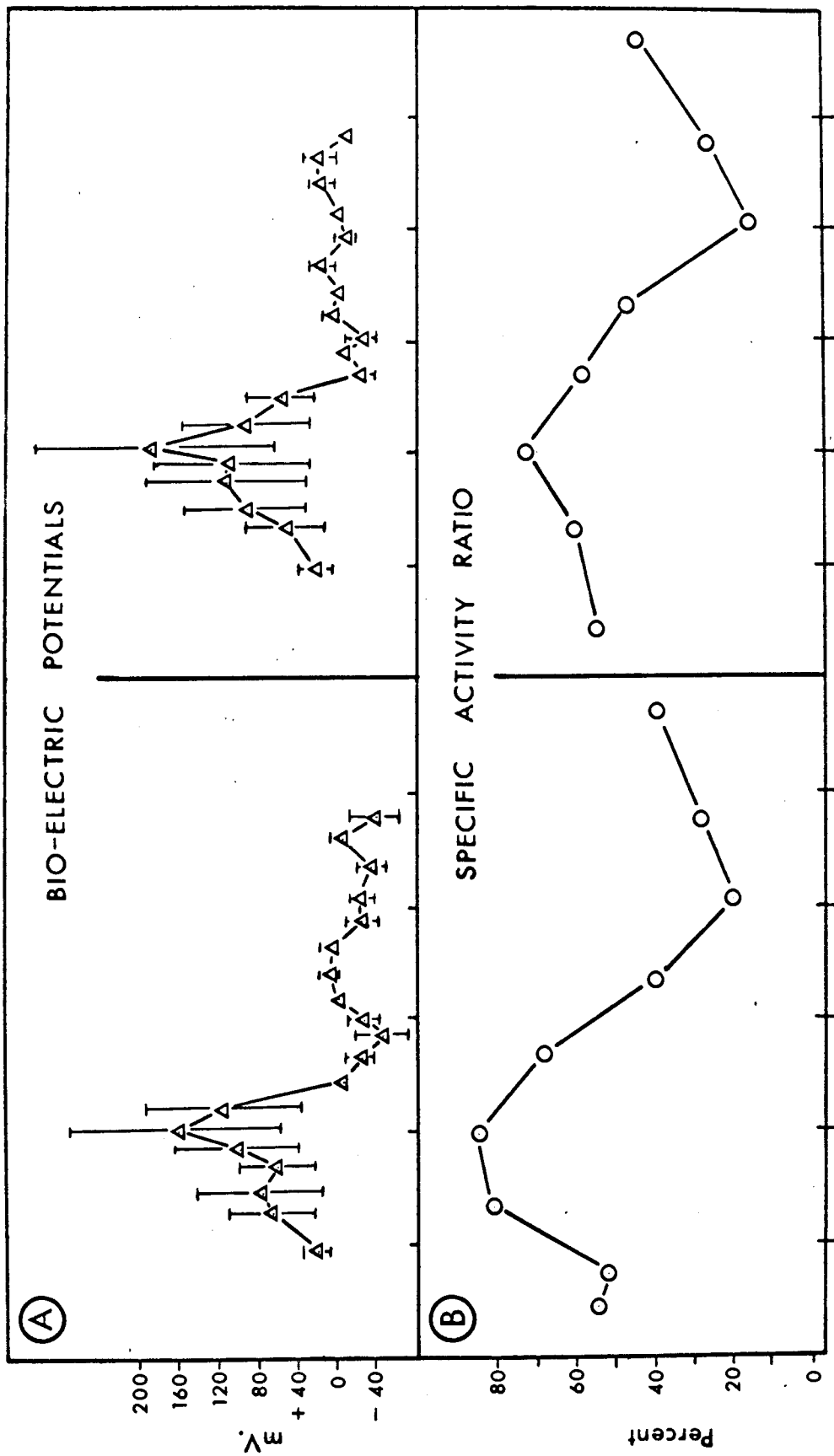

A
CROWN AIR TEMPERATURE
B
SOIL TEMPERATURE
Degrees Centigrade
0
10
20
30
M
J
J
A
S
O
M
J
J
A
S
O

Figure 5.

Seasonal changes in the bio-electric potentials, apex to base (A), and in the ratio of the specific activities of $^{14}CO_2$ respired by the shoot (B) of P. strobus L. seedlings grown under either high (HMG) or low (LMG) soil moisture conditions. Each point in A is an average value for seven seedlings and in B for two or three seedlings. B- Specific activity of $^{14}CO_2$ respired by the shoot at the end of an experiment as percentage of that immediately after the uptake of $^{14}CO_2$. Vertical bars in Fig. 5A., denote extreme maximum and minimum observations.


HMG
LMG
A
BIO-ELECTRIC POTENTIALS
mV.
200
160
120
80
+ 40
0
- 40
B
SPECIFIC ACTIVITY RATIO
Percent
80
60
40
20
0
M
J
J
A
S
O
M
J
J
A
S
O

DISCUSSION

Maintenance of constant soil-moisture conditions, at either high or low levels, did not materially change various seasonal patterns, as compared to those observed in seedlings grown under normal, variable conditions (Shiroya et al., 1966; Kienholz, 1934; Stevens, 1931). This was true for the growth of the new shoots, apparent photosynthesis of the shoot, respiration of the shoot and root, and translocation of recent photosynthate from shoot to root.

Seasonal variations in the rates of apparent photosynthesis and shoot respiration of the LMG seedlings were not significantly different from those of the HMG seedlings, as one might have expected from the reports of other workers (McGregor, 1958; Bormann, 1953; Bordeau, 1954; Negisi and Satoo, 1954; Kozlowski, 1949). A soil moisture of 9·3 per cent is at, or just below, the permanent wilting point for sunflower in a sandy loam soil (Kramer, 1949). Although pines have been shown to survive at these and even lower soil-moisture levels (Fowells and Kirk, 1945; Stone et al., 1950; and Stone, 1957), reduction in their photosynthetic rates has been recorded even at much higher moisture levels.

That the LMG seedlings, compared with the HMG ones, were definitely water stressed can be seen from the retardation of their growth and markedly lower transpiration rates, Fig. 3A. In spite of this stress the seasonal transpiration pattern of both LMG and HMG seedlings remained very similar.

During July, August and September parallel experiments were carried out on a small group of seedlings, which had their soil-moisture level maintained even lower, between 7 and 8 per cent. The results obtained from these seedlings were not distinguishable from those described above for the LMG seedlings. At this time we can offer no explanation for these apparently anomalous results.

The low moisture levels in the soil reduced the growth-rates of the new stem and needles, fresh weight and diameter of the old stem, and also the fresh weight of the roots. The rates of root growth were high in May, dropped to low values early in June, and then exhibited a second peak in the autumn. However, at the high moisture level the low rates reached after the spring peak began to increase again without any pause, while at the low moisture level they remained low for some time. As a consequence, the oncoming of their autumn peak was much more abrupt.

The high transpiration rates achieved in mid-summer approach 25 ml of water per day per seedling, which is equivalent to one to two times the total volume of these seedlings. At this time of the year more than 99 per cent of the total root surface is suberized and still such roots must be capable of high water-uptake rates. Hayward et al., (1942) and Kramer (1946 and 1965) have reported rates of water movement via suberized roots two to six times greater

than those via unsuberized ones. The reports of Chapman and Parker (1942), Reed and MacDougal (1937), and Reed (1939) indicate that trees are able to obtain adequate amounts of water independently of whether the roots are or are not actively growing and producing new unsuberized root tips.

Although, as shown in Figs. 3 and 4, the highest seasonal transpiration rate per seedling is approximately correlated in time with the maximal crown air and soil temperatures, the highest transpiration rate per unit needle surface area is not. The mid-summer peak of transpiration per unit needle surface precedes the highest air and soil temperatures by about 3 weeks. This might be either because new shoots require increased translocation of essential mineral nutrients from the roots, or because new needles lack efficient control of water loss. Ladefoged (1963) also observed high rates of transpiration during the period of maximal new shoot growth.

The autumn peak of transpiration expressed per needle stomatal surface area occurs 2-3 weeks after the maximal autumn air and soil temperatures. It is during this particular period of high transpiration rates that the majority of the previous year's needles are lost. These make up from one-third to one-half of the total needle weight in a three-year old white pine seedling, and it is possible that during the final stages of senescence they lose their control of trans-

piration loss.

As is seen in Fig. 2D there is no pronounced spring peak in the translocation of recent photosynthate to roots in LMG seedlings as reported previously by Shiroya et al. (1966). This might be due either to the effect of the low soil-moisture levels as such or to the slow recovery from the shock given to the experimental plants during their removal from the nursery beds and transplantation into pots in late April.

Two possible explanations may be given for the low translocation of recent photosynthate from shoots to roots observed in mid-summer. According to the one, this is caused by a block or discontinuity in the conducting system, which occurs at this time of the growing season. The results of Abbe and Crafts (1939) and Alfieri and Evert (1965) indicate that in late spring or early summer there may be little or even no functional phloem in the vascular bundles of at least some trees.

According to the second explanation, since the period of low translocation from shoots to roots coincides with the maximal growth of the new shoots and needles, there might be a close relationship between these two. During this period of maximal new shoot growth the hormone level in the seedling may be increased to such an extent that it begins to suppress the root growth, thus reducing the demand for the photosynthate by the root and, as a consequence, leaving this

photosynthate in the shoot. The results of Rutter (1957) and Newirth (1959) indicate that substrates are at a premium during the period of rapid shoot growth in the spring.

It was shown by Fensom (1958) that, as predicted from the electrokinetic theory of transport, when water is drawn up through the wood, positive potential differences are generated from apex to base. These are known as the streaming potentials. Since the highest seasonal bio-electric potentials observed in the present investigation occurred at the same time of the season as the highest transpiration rates per unit needle area and since the stem apex was positive with respect to the base, such potentials are probably the streaming potentials due to the transpiration stream flow. On the other hand, potentials observed at other times of the season are probably a mixture of both reduced streaming potentials and those generated by other physiological activities, such as phloem transport and electro-osmotic phenomena (Fensom, 1963).

In the course of our experiments covering several years, the precise timing of the translocation maxima and minima varied somewhat not only from one year to another, but also from one plant to another. It is very desirable, however, to time the onset and the end of this period of low translocation.

From a comparison of Figs. 2D and 5B one can see that

there is a remarkable close inverse relationship between the seasonal changes in the translocation of recent photosynthate from shoots to roots and the drop during the 8 h of the experiment in the specific activity of the $^{14}CO_2$ respired by the shoot. When translocation of recent photosynthate from shoot to root is low, the specific activity of $^{14}CO_2$ respired by the shoot during the 8 h of the experiments remains high, indicating that the relative contribution of recent photosynthate towards respiration remains about the same during the whole 8 h of the experiment. When translocation from shoots to roots is high, there is a sharp decrease in the specific activity of the $^{14}CO_2$ respired by the shoot during the 8 h of the experiment, indicating that the relative contribution of recent photosynthate towards respiration is decreasing.

The time when P. strobus seedlings are in the stage of low translocation can be determined, therefore, in the following four ways.

1. By permitting shoots to carry on photosynthesis in $^{14}CO_2$ and observing when the percentage of ^{14}C translocated to roots is the lowest.

2. By observing the growth-rates of the leader stem and the new needles. Translocation rates are at a minimum when the elongation of the leader stem is essentially complete while that of the needles is still continuing.

3. By permitting a shoot to carry on photosynthesis in $^{14}CO_2$ for 8 h and observing the magnitude of the drop in the

specific activity of $^{14}CO_2$ respired by the shoot by the end of the experiment, as compared to that immediately after the uptake of $^{14}CO_2$.

4. By observing the bio-electric potential difference between the base and top of the seedling. Translocation is at a minimum when low positive values begin to increase rapidly.

P A R T II

The Growth and Physiology of *Pinus strobus* L. Seedlings as Affected by Various Nutritional Levels of Nitrogen and Phosphorus.

INTRODUCTION

The establishment of a mycorrhizal relationship between a higher plant and a fungus has been shown to depend to a large extent on the physiology of the higher plant, which is in turn related to its environment, (Björkman, 1942, 1944, and 1949; Harley and Wade, 1955; Wenger, 1955). Björkman has also shown that the incidence of mycorrhizae is related to the levels of carbohydrates available in the roots of trees. Although the role of at least part of the soluble carbohydrate in the roots as an energy source for the fungus seems established, it has become apparent that other factors are also involved.

Correlations between the amounts of macro-nutrients e.g. nitrogen (N), phosphorus (P), potassium and calcium, in the soil and the development of mycorrhizae on tree roots has been reported by a number of workers (Björkman, 1942; Hatch, 1937, and Mitchell et al, 1937). These workers observed that low soil fertility resulted in only a few mycorrhizal associations, but with an increase in the soil fertility, though still below optimal, the number of mycorrhizae present was much higher. When soil fertility was increased further to optimal or even supra-optimal values the number of mycorrhizal associations decreased or they were totally absent. Slankis, 1964, has previously reported on the disappearance of mycorrhizae in the presence of high N level in the growth medium.

The level of available nutrients might be expected to affect the type and/or the amounts of the compounds present in the roots. This in turn may lead to quantitative and qualitative changes in the compounds exuded from the roots into the rhizosphere and thus affect mycorrhizal development. Several workers have analyzed root exudates in attempts to isolate compounds, which have either a stimulatory or inhibitary effect on mycorrhizal fungi (Melin, 1960). Slankis et al, (1964) recovered 35 radioactive compounds from the medium surrounding the roots of pine seedlings, which had fixed $^{14}CO_2$ photosynthetically for 8 days prior to the analysis of the medium. Haskaylo (1960) suggested that there must be some relationship between the amount of N and P available to a plant and the fate of its photosynthate, and that decreased translocation of sugars to roots must affect secretion of organic materials into the rhizosphere that promote the development of mycorrhizae.

The experiments reported below were carried out in order to observe the fate of recent photosynthate and the extent of mycorrhizal development in pine seedlings grown at different levels of N and P.

MATERIALS AND METHODS

White pine seedlings, Pinus strobus L., were grown in open seed-beds for 3 years at the Department of Lands and Forests Nursery, Midhurst, Ontario. From the original group of approximately 1,000 seedlings, 200 were selected on the

basis of their uniform size. In the Spring of their fourth year of growth, these selected seedlings were transplanted into individual 5 inch clay pots with 800 ml. of granitic gravel (particle size 1.19-2.00mm.), which had been washed repeatedly first with tap and then distilled water. After transplanting, seedlings were transferred to the Laboratory of Forest Pathology, Maple, Ontario, where they were kept until used for the two series of following experiments. In the series A, seedlings were kept outdoors in a special cold frame and in the series B, in controlled environment chambers.

Series A

The seedlings for these experiments were transplanted on 22nd June, and immediately placed in a special open cold frame, which had a wide roof of lathe and thin plastic sheet, 8 feet overhead, to prevent rain from falling on the seedlings. The tops of the pots were covered with styrofoam sheet through which only shoots protruded. This provided a second barrier to rain and also served to insulate the pots from direct sunlight. The light intensity at crown level was approximately 50% of the full sunlight. These seedlings were used in experiments after 13 weeks of growth at various levels of nitrogen and phosphorus.

Series B

The seedlings for these experiments were transplanted on 25 April, and were subsequently grown in controlled environmental chambers under the following conditions. The

light intensity was 2,200 ft-c at crown level provided by a combination of fluorescent (cool white) and incandescent lamps. A photoperiod of 16 hours was maintained during which the crown air temperature was 24 - 25° C. and the pot temperature 18 - 19° C. Temperatures during the dark period were 20 - 21° C. at crown level and 18 - 19° C. in the pots. Relative humidity was maintained at 75%. These seedlings were used in experiments after periods of 5 and 7 weeks of growth at various levels of N and P.

Nutrient Salt Solution

The composition of the nutrient salt solution was similar to that used by Björkman (1942). The initial pH was between 6.3 and 6.4. The final nutrient solutions, as applied to the different groups of seedlings was prepared by additions and/or deletions of various nitrogen and phosphorus containing compounds in the basic solution. Their compositions are presented in Table I.

Application of the Nutrient Salt Solution

Seedlings in both series A and B were supplied with fresh nutrient salt solution every second day. Accumulation of salts in the pots through transpiration and evaporation was prevented by flushing each pot, prior to the application of the salt solution with two aliquots of 150 ml. of distilled water. After drainage each pot was flushed with 200 ml. of fresh salt solution and allowed to drain.

The procedures and techniques for tagging the seedlings with ^{14}C, determination of the magnitude of translocation and of the rates of respiration and photosynthesis are outlined below. The selection of seedlings for use in isotope tagging experiments was initially made on a morphological basis within each experimental group immediately prior to tagging with ^{14}C. Secondly, on the basis of the individual seedling having rates of CO_2 uptake and output close to the average for that particular group under the standard experimental conditions.

At the beginning of each experiment the shoot of each seedling was enclosed separately in a plastic chamber, which was a part of a closed gas-circulating system connected to an Infra-Red CO_2 Analyzer (IRGA) and a Geiger tube. After a 1 1/2 hour induction period at 2,500 ft-c, a known amount of $^{14}CO_2$ was released into this system. Following complete uptake of the $^{14}CO_2$ by the shoot the lights were turned off and the respiration rate of the shoot was determined. The lights were then turned on again for a further 7 hours, with the seedling remaining in the closed system. At the end of the total of 8 hours from the time of the $^{14}CO_2$ release, the lights were again turned off and a second determination of the respiratory rate of the shoot was made. The seedling was then removed from the chamber and its shoot cut off at soil level. Following separation of roots from the soil,

the root respiration rate was determined. Throughout the experimental period of 8 hours, the temperatures of the shoot and roots were maintained at 25 $\pm$ 2° C and 19 $\pm$ 1° C respectively. The light source was two Silvania Photoflood lamps (type R.30, 3,400°K colour temperature) which provide an experimental light intensity of 2,500 ft-c (180 x 10^3 ergs/cm.^{2}/sec.) after filtration through 20 cm. of water.

The shoot and roots were then weighed, cut into small pieces and extracted separately in boiling 80% ethanol. Aliquots of the extracts were plated and counted. The 80% ethanol-soluble extract of the roots was separated into amino acid, organic acid and sugar fractions using resin columns, as described by Shiroya and Slankis et al. (1962). Each fraction was further resolved by two dimensional ascending paper chromatography at 20°C. The sugar and amino acid fractions were separated further using 80% phenol with 0.3% ammonium hydroxide (0.88 sp. gr.) by volume in the first direction, followed by n-propanol:ethyl acetate: water, 7:1:2 V/V developed twice in the second direction. The organic acids were separated further using ethanol (95%):water:ammonia (0.88 sp. gr.) (35:13:2 V/V) followed by ethyl acetate:acetic acid (glacial):water (50:28:25 V/V) plus sodium acetate (crystals, 120 mg/ml of the solvent) in the second direction. The chromatograms were dried and ^{14}C-containing compounds were located by

radioautography. Areas containing individual ^{14}C labelled compounds were cut from the chromatograms and assayed for their ^{14}C content in an automatic methane flow counter. Visualization for identification of the resolved compounds was accomplished by spraying replicate chromatograms with benzedine-TCA reagent to reveal the sugars, ninhydrin reagent for the amino acids and a buffered (pH 8) alcoholic solution of methyl red for the organic acids. Shikimic acid was located by spraying successively with sodium periodate and aniline solutions.

These experimental procedures were described in detail by Lister et al. (1961), Shiroya and Slankis et al. (1962), and Shiroya and Lister et al. (1966).

All values recorded in the following tables are mean replicate values from two or three seedlings analyzed separately.

TABLE I. Total final concentration of various Nitrogen and Phosphorus containing compounds in the nutrient solution as applied to the potted seedlings.

Total N and P mg/litre		mg/litre					
N	P	H_3PO_4	KH_2PO_4	$NaH_2PO_4.H_2O$	$Na_2HPO_4.12H_2O$	$(NH_4)_2HPO_4$	$(NH_4)_2SO_4$
-	173	7.9	498.7	-	628.3	-	-
-	692	7.9	498.7	1547.4	2,632.6	-	-
2.5	173	-	498.7	-	628.3	11.4	-
2.5	692	-	498.7	1547.4	2,632.6	11.4	-
53.0	173	-	498.7	-	-	245.7	4.2
53.0	692	-	498.7	1547.4	2,004.3	245.7	4.2
265.0	173	-	498.7	-	-	245.7	1,003.7
265.0	692	-	498.7	1547.4	2,004.3	245.7	1,003.7
53.0	-	-	-*	-	-	-	253.0
265.0	-	-	-*	-	-	-	2,003.2

*Potassium content provided by adding 273.1 mg/litre of KCl.

RESULTS AND DISCUSSION

The effects of various nutritional levels of nitrogen (N) and phosphorus (P) on Pinus strobus L. seedlings grown for 13 weeks in cold frames (series A) are shown in tables II - IX. Table II shows that the highest weight of the whole seedling occurred at the intermediate levels of both N and P (N-53 mg/l, P-173 mg/l). At each P level with increasing N there was, as a rule, an increase in the shoot fresh weight and in the average length of the new needles (Tables III and IV). However, at the same level of N an increase in P resulted first in an enhanced shoot fresh weight and then in an inhibition. These results are in general agreement with those of Mitchell et al. (1937) and Mitchell (1939) who found that for the white pine seedlings the optimal concentration for N was approximately 300 mg/l and for P, 350 mg/l.

The highest weight of roots (Table V) and the highest root/shoot ratio (Table VI) also occurred at the intermediate levels of N and P. Mitchell (1939) found that the root/shoot ratio was directly proportional to the P concentration up to the inhibiting levels and inversely proportional to the N concentration above 30 mg/l. He also showed, however, that the shoots show a greater response to an increase in N than do roots, while the situation was reversed with respect to P. One might therefore expect best over-all plant growth at some intermediate level of both N and P, as is illustrated in tables II and VI and plate I.

Plate I, shows the over-all appearance of seedlings under various nutritional levels and the proportion of main roots to secondaries. At high concentrations of N and especially of P the number of secondary roots decreases and the main roots are somewhat shorter and thicker. Mitchell (1939) found similar changes and also noted a parallel reduction in the number of root hairs.

Table VII, shows the relative abundance of mycorrhizae on the roots of experimental seedlings. Mycorrhizae were present on all seedlings receiving below optimal levels of N and P, but were more abundant on seedlings receiving intermediate levels of both N and P. A large imbalance between N and P supplied resulted in a decreased incidence of mycorrhizae independent of whether it was N or P that was deficient. Mitchell at al. (1937) reported similar observations and observed the highest frequency of mycorrhizae to be on those seedlings which had the highest root/shoot ratios. He concluded that their results agreed in every respect with the Stahlian-Hatch theory of the relationships between mineral salts and mycorrhizal formation.

The highest rates of apparent photosynthesis (Table VIII) were observed at the intermediate levels of both N and P. There was no discernable effects of the levels of N & P nutrition on the respiration of either roots or shoots.

Translocation of the recent photosynthate from shoots to roots tended, on the whole, to be the highest at the inter-

mediate levels of both N & P (Table IX). At the same level of N an increase in P always decreased translocation to roots. The effect of an increase in N depended on the level of available P, at high P levels there was an increase and at intermediate or low P a decrease.

Since it was found from previous experiments that between 85-90% of ^{14}C translocated from shoots to roots in 8 hours was present in the ethanol-soluble fraction (Shiroya et al., 1966), distribution of ^{14}C compounds in the roots has been examined in this fraction only.

Table X shows distribution of ^{14}C among the sugar, amino acid and organic acid fractions. A rise in the levels of N at all P levels increased the percentage of ^{14}C recovered in both amino and organic acids and decreased the percentage in the sugars. Since the relative amounts of sugars were large, and those of amino acids and organic acids small, even a small decrease in the percentage of sugars (from 99 to 95%) resulted in a very large increase in the absolute amounts of amino and organic acids. Thus at 0 mg/l of P an increase in the level of N nutrition brought about an increase in the amino acid fraction from 1.31 to 1.50 or by 15%; at 173 mg/l of P from 0.08 to 1.47 or by 1,800% and at 692 mg/l of P from 0.05 to 2.10 or by 4,200%. A similar effect is observed also for the organic acid fraction.

The distribution of ^{14}C among the various compounds of these three fractions is shown in Tables XI, XII, and XIII.

As is seen from Table XI, sucrose was always the main sugar found in roots. It was reported earlier by Shiroya and Lister et al. (1962) that in white pine, sucrose was the main compound in which photosynthetically assimilated carbon is translocated from shoots to roots. Table XI also shows that an increase in N at all P levels resulted in an increased hydrolysis of sucrose. At the same level of N an increase in the level of P nutrition raised the recovery of ^{14}C in sucrose. The highest amounts of ^{14}C in raffinose were found at zero P.

Table XII presents data on the distribution of ^{14}C in the ethanol-soluble amino acids found in the roots. α-Alanine, aspartic acid, glutamine, glutamic acid + serine, glycine and tyrosine and/or β-amino butyric acid were the main recipients of ^{14}C. High N increased recovery of ^{14}C in some compounds e.g. in glycine, but decreased it in others e.g. in aspartic and glutamic + serine, tyrosine and/or β-amino butyric. High levels of N not only increased the recovery of ^{14}C in this fraction but also increased the total amount of free amino acids present, whether labelled or not. For example, the amounts of Arginine (+) increased tenfold while only in one instance were they found to contain ^{14}C. The increases observed among some components of the amino acid fraction are similar to those found by Cocking and Yemm (1961) to be typical of the metabolism of roots of plants grown under high N levels.

The levels of P nutrition had no clear effects on the distribution of carbon within this fraction.

Table XIII shows distribution of ^{14}C within the organic acid fraction in the roots. Approximately 50% of ^{14}C was found among the "12 compounds", which have remained unidentified. The effects of changing nutritional levels of N and P on the distribution of ^{14}C among various organic acids were variable. With increasing levels of either N or P some compounds showed an increase in their ^{14}C content, e.g. glycolic acid, others a decrease, e.g. malic and succinic acids.

The data obtained from the series B, both after 5 and 7 weeks of growth in a growth chamber, was similar to those described above for the series A (seedlings grown for 13 weeks in cold frames). The main difference was that the trends described above were less pronounced. As is shown in Plate II, shoot growth increased under the influence of increasing N nutrition, and the roots possessed fewer secondary roots even after 5 weeks on treatment. The maximum relative abundance of mycorrhizae was again observed on seedlings receiving intermediate levels of N and P, which correlated with the seedlings having the highest root/shoot ratios.

It is of interest to compare the observed range of soluble compounds found in the roots with those exuded into the growth medium from the roots of white pine seedlings, as reported by Slankis et al. (1964). The last investigators found 35

different compounds exuded by the pine roots and identified the following: glucose, arabinose, asparagine, glutamine, malic, oxalic, malonic, glycolic, shikimic and cis-aconitic acids. Eriksson (1960) and Melin (1960) have also reported the presence of vitamins and several amino acids, particularly glutamic acid and aspartic acid, in pine root exudates. In the present investigation, glucose, glutamine, malic and glycolic acids not only were found in the roots, but judging from their high ^{14}C content were very active metabolically. Asparagine, though present, was apparently less active metabolically. Shikimic acid, present in considerable amounts, was seldom labelled with ^{14}C during the 8 hour experimental period. Malonic, oxalic and cis-aconitic acids and arabinose were not found. No clear-cut correlation could be observed between the incidence of mycorrhizae on the roots and changes in the ^{14}C content of any single sugar, amino acid or organic acid brought about by different levels of N or P.

The results reported here are in general agreement with the work and ideas of both Björkman and Hatch on the formation of the mycorrhizal association. Björkman (1942) found a close correlation between the carbohydrate levels in the roots and mycorrhizal abundance. Both Björkman (1949) and Wenger (1955) showed that the number of mycorrhizae increased with the amount of light the host plant received and suggested that increased light leads to increased photosynthesis and trans-

location of photosynthate to roots. Shiroya and Lister et al. (1962) actually have shown that white pine seedlings grown under high light intensity translocate more of their recent photosynthate to the roots than those grown under low light.

Hatch (1937) found that when the host plant is moderately deficient of N and P, to the extent that its growth is somewhat retarded, there is an accumulation of carbohydrate in the plant and many mycorrhizae are found on its roots. In the present investigation mycorrhizae were also found to be most abundant at the intermediate levels of N and P nutrition when the sucrose translocated from shoots to roots was metabolized into a variety of various substances. Therefore, among the root metabolites of healthy vigorous plants, there are probably compounds which are essential to the mycorrhizal fungi and for mycorrhizal formation, as well as an abundant supply of sugars. Conditions of high P inhibit the metabolism of the translocated sugars to some extent and both the type and amounts of compounds resulting from such metabolism is changed. High N increases the utilization of sugars by the host plant itself, reducing the amount available to the fungus as was found also by Björkman (1942). The high recovery of ^{14}C reported here in the reducing sugars under high N conditions does not necessarily mean that they are present in considerable amounts, only that the turnover is both rapid and at the expense of recently translocated photosynthate. However, no definite correlation has been found between the

number of mycorrhizal associations and the concentration of any particular substance in a root. It is possible, therefore, that the formation of mycorrhizal associations may be determined not by the concentration of any single substance but by a group of them present in definite absolute and relative amounts.

There may also be initiated what can be looked upon as feed-back systems. For example, the effect of P, which Mitchell (1939) showed to have a greater effect on roots than shoots, could well be via the increased absorption capacity of mycorrhiza for P as observed by McComb (1943) and McComb at al. (1946). The increase in available P results in enhanced root growth which in turn increases the number of potential mycorrhizal sites and the circle is complete. A similar but reverse effect may take place under conditions of high N which is seen to result in a reduction of lateral roots and hence the number of potential mycorrhizal sites.

The data presented here shows that there is a relationship between availability of N and P within the plant and the disposition of the photosynthate. Translocation of recent photosynthate to the roots is inhibited by high P concentrations but this inhibition can be reversed, within limits, by increasing the N concentration, that is by reducing the imbalance of nutrients. However, correlations of the data with the ratio of N to P cannot be made due to the influence that

the presence or absence of mycorrhizae may have on the availability of nutrients to the seedlings.

It would appear that the level of sugars in the plant is related to the release of compounds into the rhizosphere on two counts, first, by provision of an energy source for metabolism and secondly, by provision of a carbon skeleton for synthesis of a varied array of organic compounds. The changes brought about by the differing availability of N and P may be in part through their effect on promoting the use of the sugars within the shoot and hence reducing the amount translocated to the roots, but also, and probably more important with respect to the mycorrhizal system, by determining the metabolic fate of the sugars once they have arrived in the root.

The mycorrhizal system appears to be one of limited symbiosis and not one of controlled parasitism as suggested by Burgess (1936). Under very infertile conditions neither the higher plant partner nor the fungus survives, when nutritional conditions are in the intermediate range, as in the majority of cases in nature, both survive and there may be a limited symbiotic relationship. Under optimal conditions for the higher plant, the relationship is reduced, probably due to the changed metabolism of the higher plant.

The reduction in number or incidence of mycorrhizae under favourable conditions for growth of the higher plants, e.g. in fertile agricultural soils, could be due in part to the form of the root of the higher plant. If the number of

potential mycorrhizal sites is limited by a reduction in the occurrence of lateral roots, then the number of mycorrhizae will be lower and the system is therefore under the control solely of the higher plant partner. On the other hand, it may be found that the percentage of potential mycorrhizal sites actually occupied by mycorrhizal structures is also reduced, in which case part of the control must be directly via the rhizosphere environment.

TABLES II - IX

The effects of various nutritional levels of nitrogen and phosphorus on *P. strobus L.* seedlings

TABLE II. Total seedling fresh weight. (g).

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	42.6, 35.6 } 39.1	30.5, 23.5 } 27.0
2.5	-	29.2, 45.0 } 37.1	17.5, 26.5 } 22.0
53.0	36.0, 33.8 } 34.9	67.7, 56.0, 58.1 } 60.6	28.2, 25.8 } 27.0
265.0	32.9, 28.7 } 30.8	46.2, 41.5 } 43.9	34.0, 27.5 } 30.7

TABLE III. Shoot fresh weight. (g).

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	11.0, 15.2 } 13.1	10.5, 7.0 } 8.7
2.5	-	8.2, 12.0 } 10.1	10.5, 9.5 } 10.0
53.0	10.5, 11.2 } 10.8	17.7, 18.0, 17.1 } 17.6	11.7, 11.3 } 11.5
265.0	11.4, 11.2 } 11.3	17.5, 18.2 } 17.9	17.5, 13.0 } 15.2

TABLE IV. Average new needle length (cm).

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	4.5, 3.8 } 4.2	3.9, 3.7 } 3.8
2.5	-	3.4, 3.2 } 3.3	4.4, 4.6 } 4.5
53.0	3.0, 4.6 } 3.8	4.5, 4.1, 5.0 } 4.5	3.7, - } 3.7
265.0	4.5, 5.1 } 4.8	5.9, 4.4 } 5.2	5.8, 4.7 } 5.3

TABLE V. Root Fresh weight. (g).

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	27.4, 24.6 } 26.0	20.0, 16.5 } 18.2
2.5	-	21.0, 33.0 } 27.0	7.0, 17.0 } 12.0
53.0	25.5, 22.6 } 24.0	38.0, 41.0, 50.0 } 43.0	16.5, 14.5 } 15.5
265.0	21.5, 17.5 } 19.5	24.0, 28.0 } 26.0	21.0, 10.0 } 15.5

TABLE VI. Root/Shoot ratio (on fresh weight basis).

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	1.80, 2.23 } 2.01	1.90, 2.36 } 2.13
2.5	-	2.56, 2.75 } 2.70	0.67, 1.70 } 1.19
53.0	2.01, 2.42 } 2.22	2.82, 2.11, 2.39 } 2.44	1.41, 1.28 } 1.35
265.0	1.88, 1.56 } 1.72	1.53, 1.37 } 1.45	1.61, 0.57 } 1.09

TABLE VII. Mycorrhizal abundance (Arbitary units).

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-(1)	++	++
2.5	-	++++	++
53.0	+	+++	+
265.0	+	0(2)	0

(1) - , not determined

(2) 0, mycorrhizae absent

TABLE VIII. Apparent photosynthesis ($mgCO_2/hr/g.fr.wt.$)

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	0.26, 0.59 } 0.43	0.25, 0.40 } 0.32
2.5	-	0.74, 0.50 } 0.62	0.30, 0.25 } 0.28
53.0	0.42, 0.28 } 0.35	1.02, 0.48, 0.37 } 0.62	0.51, 0.49 } 0.50
265.0	0.31, 0.38 } 0.35	0.36, 0.40 } 0.38	0.44, 0.34 } 0.39

TABLE IX. Translocation

Level of N treatment (mg/l)	Level of P treatment (mg/l)		
	0.0	173	692
0.0	-	11.6, 10.4 } 11.0	3.8, 3.2 } 3.5
2.5	-	2.7, 15.3 } 9.0	2.4, 8.0 } 5.2
53.0	7.4, 19.4 } 13.4	11.5, 10.5, 15.7 } 12.6	6.5, - } 6.5
265.0	11.3, 11.2 } 11.2	9.2, 12.8 } 11.0	3.7, 11.8 } 7.7

The amount of ^{14}C recovered in the 80% ethanol-soluble fraction of the root as a percentage of the total ^{14}C absorbed as $^{14}CO_2$ by the seedling.

TABLE X. Percentage distribution of ^{14}C, translocated from the shoot, among the 80% ethanol-soluble compounds of the roots of P. strobus L. seedlings grown under various levels of nitrogen and phosphorus nutrition.

	Levels of P treatment (mg/l)									
	0.0		173				692			
	Level of N treatment (mg/l)		Level of N treatment (mg/l)				Level of N treatment (mg/l)			
	53	265	0.0	2.5	53	265	0.0	2.5	53	265
Sugars	98.20	97.90	99.52	99.55	99.19	96.50	99.80	99.14	98.73	95.40
Amino Acids	1.31	1.50	0.08	0.06	0.36	1.47	0.05	0.16	0.52	2.10
Organic Acids	0.49	0.60	0.40	0.39	0.45	2.03	0.15	0.70	0.75	2.50

Each value is the mean of 2 or 3 seedlings analyzed separately.

TABLE XI. Percentage distribution of ^{14}C, translocated from the shoot, among the various components of the sugar fraction from roots of P. strobus L. seedlings grown under various levels of nitrogen and phosphorus nutrition.

	Level of P treatment (mg/l)									
	0.0		173				692			
	Level of N treatment (mg/l)		Level of N treatment (mg/l)				Level of N treatment (mg/l)			
	53	265	0.0	2.5	53	265	0.0	2.5	53	265
Sucrose	55.0	53.0	85.4	78.8	71.1	57.0	82.6	91.4	86.2	70.8
Glucose	14.2	10.9	4.8	7.4	12.1	17.2	4.9	2.9	5.7	8.6
Fructose	13.5	11.0	4.7	7.0	10.8	16.3	4.5	2.6	5.1	8.1
Raffinose	14.9	23.2	4.7	5.8	4.8	6.9	7.7	2.8	2.4	11.3
Unknown 1	2.4	1.9	0.4	1.0	1.2	2.6	0.3	0.3	0.6	1.2

Each value is the mean of 2 or 3 seedlings analyzed separately.

TABLE XII. Percentage distribution of ^{14}C, translocated from the shoot among the various components of the amino acid fraction from roots of P. strobus L. seedlings grown under various levels of nitrogen and phosphorus nutrition.

	Level of P treatment (mg/l)									
	0.0		173				692			
	Level of N treatment (mg/l)		Level of N treatment (mg/l)				Level of N treatment (mg/l)			
Compound	53	265	0.0	2.5	53	265	0.0	2.5	53	265
α Alanine	6.6	10.9	25.4	14.6	23.5	18.9	18.8	13.6	34.0	11.3
β Alanine	0.9	0.2	1.9	+	+	+	+	+	3.4	8.7
Asparagine	+**	+	+	+	Tr	10.4	0*	5.4	1.0	4.5
Aspartic	8.2	2.7	13.1	21.3	5.5	8.4	28.1	7.8	10.2	4.3
Glutamine	7.5	3.2	3.0	Tr	7.3	20.2	+	7.3	5.0	26.8
Glutc-Serine (1)	20.4	11.0	26.3	45.8	41.9	14.3	43.7	41.5	29.7	15.3
Glycine	51.5	65.1	2.3	3.1	2.6	17.8	+	+	4.4	12.0
Threonine	0*	0	0	0	+	+	0	0	0	+
Leucine (+) (2)	0.3	1.5	+	Tr	2.8	2.2	+	+	2.7	4.5
Methionine	1.0	1.5	+	Tr	5.4	1.3	+	+	1.0	3.8
Phenyl alanine	0	0	0	0	+	+	0	0	0	+
Proline	+	0.4	+	+	+	+	+	+	1.0	2.0
Trypt./δ am.b (3)	+	0.3	+	+	+	+	0	+	0	2.3
Tyr./β am.b (4)	3.6	2.6	28.0	15.2	11.0	4.5	9.4	24.4	4.7	4.5
Valine	Tr***	+	0	+	Tr	2.0	0	+	2.9	0
Arginine (+) (5)	++	0.5	0	0	+	++	0	0	+	++
Cysteine-cystine	0	+	0	0	+	Tr	+	0	0	0

Each value is the mean of 2 or 3 seedlings analyzed separately.

(1) Mainly glutamic acid.

(2) Leucine complex, not further resolved.

(3) Tryptophane and/or δ amino butyric acid.

(4) Tyrosine and/or β amino-butyric acid.

(5) Basic amino acids, mainly Arginine

*0, absent, as determined by ninhydrin test. **+, present, as determined by ninhydrin test, but containing no ^{14}C. ***Tr, trace of ^{14}C (0.1%).

TABLE XIII. Percentage distribution of ^{14}C, translocated from the shoot, among the various components of the organic acid fraction from roots of P. strobus L. seedlings grown under various levels of nitrogen and phosphorus nutrition.

	Level of P treatment (mg/l)									
	0.0		173				692			
	Level of N treatment (mg/l)		Level of N treatment (mg/l)				Level of N treatment (mg/l)			
Compound	53	265	0.0	2.5	53	265	0.0	2.5	53	265
Citric	6.3	4.8	5.7	6.2	4.8	2.7	3.8	4.5	5.9	2.6
∝ Ketoglutaric	6.4	10.0	5.3	8.1	1.1	9.0	3.4	6.0	5.0	4.9
Succinic	7.1	4.5	2.6	7.9	4.0	1.1	4.1	5.5	2.3	1.1
Malic	13.7	11.7	18.1	28.4	18.6	9.9	19.9	19.0	13.6	7.1
Fumaric	0*	+**	0	0.3	0.4	0	+	0.3	0	0
Glyceric	0	13.2	0	0	0.3	0	+	0	0.3	1.7
Glycolic	12.2	12.3	4.7	1.1	9.6	40.8	0.7	1.7	3.6	33.5
Glyoxylic	0	+	0	0	+	Tr****	0	+	+	+
Shikimic	6.0	+	+	+	+	+	+	+	+	+
Unknown 1	6.0	19.8	5.8	7.1	4.5	7.6	+	6.6	14.0	14.0
2	4.9	-	3.3	1.1	4.5	2.0	-	-	2.1	1.1
3	2.4	Tr	1.6	+	+	1.1	+	0	0.6	1.2
4	18.3	3.9	22.2	4.8	0.4	-	7.5	11.7	0.7	1.2
5	3.1	-	3.0	-	-	-	-	-	-	-
6	12.0	15.2	23.5	26.4	29.6	21.0	36.8	33.0	43.6	23.7
7	1.6	2.8	4.2	4.0	11.5	3.7	9.1	2.6	5.0	6.7
9	-***	-	-	0.7	9.1	1.1	6.6	-	2.1	0.7
10	-	-	Tr	3.6	0.9	-	8.1	9.1	1.2	0.5
11	-	1.8	-	0.3	0.3	-	-	-	-	-
12	-	-	Tr	Tr	0.4	-	-	-	-	-
Total Unknown	48.3	43.5	63.7	48.0	61.2	36.5	68.1	63.0	69.3	49.1

Each value is the mean of 2 or 3 seedlings analyzed separately.

*0, absent, as determined by spray test.

**+, present, as determined by spray test, but containing no ^{14}C.

***-, these compounds were not detected by the spray tests applied.

****Tr, trace of ^{14}C (<0.1%).

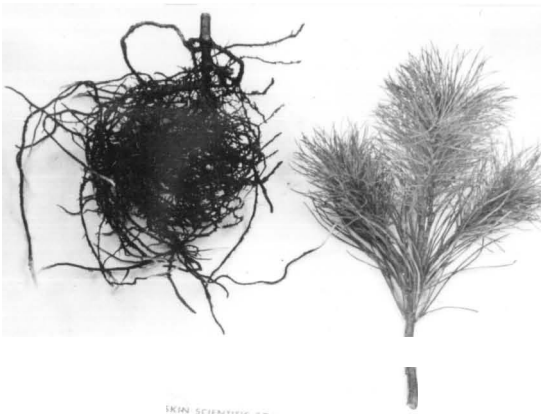
PLATE I

The appearance of *P. strobus L.* seedlings after a period of 13 weeks growing in a cold frame under various levels of nitrogen and phosphorus nutrition.

A. 0 mg N/l., 173 mg P/l
B. 53mg N/l., 0 mg P/l
C. 2.5 mg N/l., 173 mg P/l
D. 53mg N/l., 173 mg P/l
E. 265 mg N/l., 173 mg P/l
F. 53mg N/l., 692 mg P/l

A, C, D and E, illustrate the effect of increasing nitrogen concentration with 173 mg P/l.

B, D and F, illustrate the effect of increasing phosphorus concentration with 53 mg N/l.



A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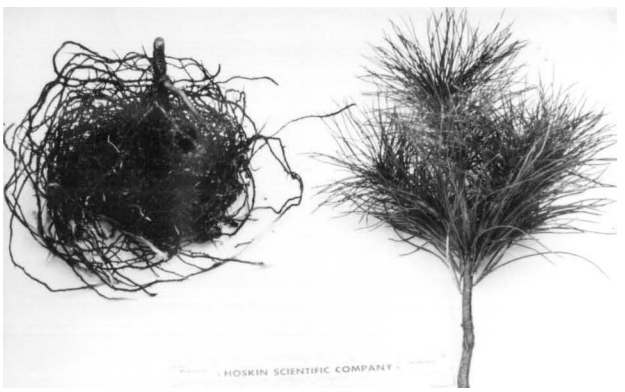
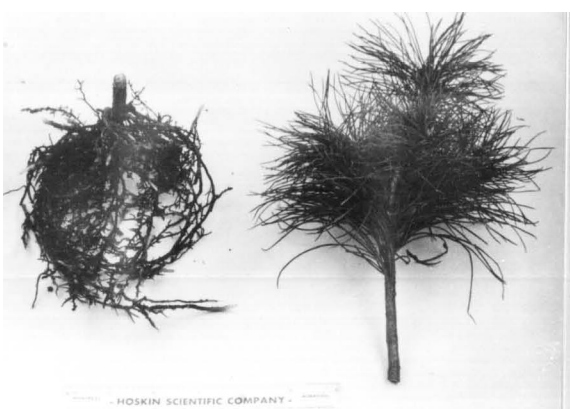
B

C


HOSKIN SCIENTIFIC COMPANY

D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E


HOSKIN SCIENTIFIC COMPANY

F

PLATE II

The appearance of P. strobus L. seedlings after five and seven weeks in a growth chamber under low or high nitrogen and constant phosphorus nutritional levels.

A. After five weeks on low N (0 mg N/l).

B. After seven weeks on low N (0 mg N/l).

C. After five weeks on high N (265 mg N/l).

D. After seven weeks on high N (265 mg N/l).

Phosphorus nutritional level in all cases was 173 mg P/l.


HOSKIN SCIENTIFIC COMPANY
C -N (II)

A

B


B. N5 (I)


HOSKIN SCIENTIFIC COMPANY
C N5 (II)

C

D

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EPILOGUE:

Directions for Future Research

The development and combination of various techniques has evolved into a basic experimental system which has facilitated the gaining of some increased insight into the physiology of one species of conifer, Pinus strobus L.

Basic information of this type is necessary before detailed studies on the fate of assimilated carbon can be undertaken. The nature and metabolism of the wide array of compounds which make up wood are of prime concern to the Forest Products Industry. Data as to how the various environmental factors may affect the make up of wood could lead to selection of timber from specific habitats for use in specialized applications, thus increasing the efficiency of utilisation.

Information on the movement of natural products throughout the tree is an essential pre-requisite to studies concerned with the control of the many diseases and insect predators which result in such a tremendous loss of timber at the present time. Once the pathways and control system(s) of the movement of natural products is more clearly understood, the movement, distribution and metabolic fate of synthetic pest control chemicals introduced into trees can be definitively studied.

The application of this basic experimental system and the insights gained in the rather limited natural environment of Southern Ontario could now be profitably applied to the study of other economically important species, not only on material grown under controlled conditions, but also upon more mature trees in their complex and diversified habitats of the Pacific Northwest.

CURRICULUM VITAE

Name: Geoffrey Richard Lister

Place and year of birth: Harworth, Yorkshire, England, 1934

Education:

Rotherham Grammar School 1945-1953
School Certificate - 1950
G.C.E. (Advanced Level) - 1952
G.C.E. (Scholarship Level) - 1953

University of Liverpool, 1953-1957
B.Sc., 1956
B.Sc. (Hons. Bot.), 1957

Simon Fraser University
Graduate Studies, 1965-1967

Experience:

Team Leader - Research Projects
Marine Biological Station,
Port Erin, Isle of Man. 1956 and 1957.

Laboratory Demonstrator, Dept. of Biology, Queen's University, Kingston, 1959-1961.

Research Assistant to Prof. G. Krotkov, Dept. of Biology, Queen's University, Kingston, 1959-1962.

Research Associate, Dept. of Biology, Queen's University and Dept. of Agriculture and Forestry, 1963-1965.

Teaching Associate, Dept. of Biological Sciences, Simon Fraser University, Burnaby, B.C., 1965-1967.

Publications:

1. Lister, G.R., Krotkov, G. and Nelson, C.D., 1961. A closed circuit apparatus with an infra-red CO_2 analyzer and a geiger tube for continuous measurement of CO_2 exchange in Photosynthesis and respiration. Can. J. Bot. 39: 581-591.

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Papers presented to Scientific Societies:

A. Canadian Society Plant Physiologists
1. 1962, Laval University, Quebec City.
2. 1963, University of Manitoba, Winnipeg.
3. 1965, University of New Brunswick, Fredericton.

B. American Society of Plant Physiologists
1. 1963, University of Massachusetts, Amherst.
2. 1965, University of Illinois, Urbana.

Professional Society Membership:

Canadian Society of Plant Physiologists (full member).

American Society of Plant Physiologists (full member).