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GROWTH OF WHITE PINE SEEDLINGS IN RELATION TO AVAILABLE CARBOHYDRATES

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Charles E. Darrell
1940

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IN RELATION TO AVAILABLE CARBOHYDRATES

by

Charles E. Darrell

A THESIS

Submitted to the Graduate School of Michigan
State College of Agriculture and Applied
Science in partial fulfilment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Forestry

1940

ACKNOWLEDGMENT

The author wishes to take this opportunity to express his appreciation to Professor M. E. Deters for the unlimited cooperation and assistance given by him in the performance of this work, and to Professors R. P. Hibbard and H. C. Beeskow for their advice and assistance and for making available the equipment of the Botany Department laboratories.

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INTRODUCTION

Some forest tree seedlings, when planted in the field, become established rapidly and grow vigorously. Other seedlings fail to become established or grow very slowly despite reasonably favorable growing conditions. Nurserymen therefore often classify planting stock into several grades, attempting to cull the inferior, low vigor stock--those which would not survive if planted in the field. The criteria commonly used in culling practices are: size of stock, top-root ratio, length and color of leaves and general appearance. Seedlings from a dominant position in the seedbed usually develop as select stock while suppressed seedlings or those grown very densely are second grade or cull stock.

While the external characteristics of seedlings are of great value in determining the quality of the stock, there must be a physiological basis for the differences which exist. Since stored food reserves have an important influence upon plant growth, it was considered that the available carbohydrate content of tree seedlings might provide a satisfactory measure of growth following transplanting.

The study was made using white pine seedlings of from one to four years of age and of three quality classes except for the one year seedlings in which only two quality classes were obtainable.

HISTORY

Research in carbohydrate content of trees and their influence on various tree organs and physiological activity is generally limited to a few species and has been in progress a comparatively short time. Especially is this true where forest trees are concerned. This latter condition can be attributed to the fact that forestry as a science is of recent date, particularly in America.

Graber, et al.,(8), in their work on organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants conclude that 1. Storage of organic food reserves in roots takes place during the fall dormancy period, 2. Some loss of food reserves in roots occurs during winter and before growth starts in spring, and 3. The maturity and amounts of top growth, and the longevity of the alfalfa plants were very generally associated with a high content of carbohydrate and nitrogen reserves in the root.

McCarty(16), working with Elymus ambiguus, Vasey and Scribn., and Muhlengergia gracilis, (Nutt.)Hitch., states, "In general, the trend of carbohydrates are from low concentration in the early part of the growing season to high concentration, the maximum occurring during the declining phase of growth. The greatest change is in the starch fraction, while there are only slight changes in the reducing sugars. There is a decline in the hemicellulose concentration during time of flowering and development of fruit." Seasonal march of carbohydrates was found to be in inverse ratio to the rate of growth of the herbage.

Mitra(17), in his work on two year old seedlings of apple, concluded that total carbohydrates increase rapidly in stem and roots at the close of the growing period in August, September, and October. From October through December there is a gradual decrease, followed by a rapid decrease in January, February and March, when the minimum is reached. The translocation sugars, glucose and maltose, are most abundant during the dormant season, increasing rapidly in stems during the early spring.

This increase in sugars during the winter, followed by a decrease in spring and early summer, was also noted in conifers by Dixon and Atkins(5),in Picea canadensis,L., Linnaea borealis, Gronov., and Pyrola rotundafolia, L., by Lewis and Tuttle(14), in some western evergreens by Gail(6), in apple spurs by Hooker(9), and in spruce by Goldsmith and Harris(7). Preston and Phillips(24) state that "There is no great increase in the content of sugars in stems and roots, except in spring as the buds unfold," and "The maximum for total carbohydrate reserves for deciduous leaved trees is at the time of leaf fall in autumn, whereas the maximum is at the opening of buds in the spring for persistent leaved trees."

Abbott(1) describes what he terms a "rest period" in apple and peach trees occurring between July and October in which there is no response to growing conditions. He points out that accumulation of carbohydrates and the beginning of the rest period are associated phenomena.

In their treatise on The Physiology of the Apple, Butler, Smith, and Curry(4) state that the supply of carbohydrate material needed by the growing parts is mainly furnished by the hydrolysis of the saccharase stored in the small roots. This statement is not substantiated by others who assume that the necessary sugars are furnished by hydrolysis of starch.

Traub(25) found for 2-3 year portions of apple twigs that the minimum in reserve carbohydrates is reached during April and May, just before inception of growth.

Hemicelluloses may be regarded as a reserve carbohydrate supply, Murneek(21). They occur primarily as thickenings of the cell wall, and are made up chiefly of dextrosans, mannans, galactans and pentosans.

Hemicelluloses may be hydrolyzed, at least in part, though probably not completely, to the corresponding sugars. This is substantiated by Jones and Bradlee's(10) work on maple trees. They state that sucrose and hexose increases and starch decreases during the winter season are always associated with a decrease in hemicellulose content.

In evergreens, roots and stems are not the only places of starch storage. Miyake(20) points out that starch in evergreen leaves, generally speaking, begins to decrease in November, reaching its minimum during January and the beginning of February, and increases again from the end of February. The starch content of evergreen leaves is generally more abundant in spring than in late summer or early autumn.

Langlet(12) concluded from extensive experimenting that the sugar content of one year pine seedlings as well as needles of older plants are low in the fall, high during the winter, and low again in the spring.

The disappearance of the soluble carbohydrates in the spring is probably due either to their reconversion to insoluble carbohydrates, to their rapid utilization in early spring growth, or both, Meyer(17).

In general, then, reserve or stored carbohydrates in perennial plants are in greatest amounts in roots, stems, and leaves in early fall. They decrease gradually until inception of growth in spring then rapidly until a minimum is reached just before the beginning of rapid photosynthetic activity. Although there are periodic fluctuations in amounts of both sugars and polysaccharides, the greatest changes occur in the latter.

Hemicellulose as well as starch is a source of sugars in plants. These are drawn upon even though sufficient quantities of starch are present.

In conifers, carbohydrates are stored in the needles as well as in roots and stems.

FIELD PROCEDURE

White pine, Pinus strobus, L., of 1-0, 2-0, 3-0, and 4-0 stock grown in the Michigan State College forest nursery was selected and used for the experiment.

All seedlings were lifted on April 15, 1939, before active spring growth started. Each age group was divided into three grades: A, select; B, average; and C, poor. The grade in which a seedling fell was determined by size of stem, amount of foliage and relative vigor as determined by general appearances.

Twenty trees of each grade were lifted, care being taken to save as much of the root system as possible. Each seedling was tagged immediately upon lifting and placed in wet burlap to prevent drying of root hairs.

Ten trees of each grade were healed-in and planted the following day, April 16, 1939 in a well prepared nursery bed. The soil was well-drained, gravelly, sandy loam. Rows were one foot apart and seedlings within the rows were set at ten-inch intervals to allow sufficient space for growth. Throughout the growing season the bed was watered frequently to maintain favorable soil moisture content. These methods of spacing and watering eliminated any important effects from competition. Weeds and grass were kept out of the area during the growing season.

Measurements of the season's growth were made to the nearest one-tenth inch after the 1939 growing season had ended. Two separate sets of measurements were taken: 1) Height growth --growth of the terminal shoot and 2) Total growth--sum of growth of all terminal and lateral shoots.

LABORATORY PROCEDURE

On the day that the trees were lifted, April 15, 1939, ten seedlings of each grade were prepared for laboratory analysis. They were carefully washed to remove all dust and dirt, cut into small pieces to facilitate drying, and placed in an electric oven. The temperature was kept at 100°C for half an hour and then dropped to 65°C until complete drying of samples. The high initial heat was used to stop all enzymatic action.

The samples were dried to a constant weight after having been in the oven for 26 days. After the seedlings were dried and weighed, weights being taken to the nearest miligram, they were ground to a fine powder. In each grade three separate samples of the ground material were taken for carbohydrate determination.

Carbohydrate determination.

The powdered seedling samples were placed in 400cc. of 80% alcohol and boiled in a reflux condenser for one hour. The liquid was decanted and the process repeated. In order to separate the soluble from the insoluble carbohydrates, the liquid and residue were poured into a folded filter paper, allowed to filter, and washed thoroughly with warm 80% alcohol. The filtrate then contained all the soluble matter such as the mono- and di-saccharides, lipoidal material, mineral matter, etc. The residue contained the soluble and insoluble starches, dextrines, and hemicellulose besides lignin, cellulose, complex proteinaceous matter and other complex substances.

Soluble or reducing sugars.

The filtrate was transferred to a large, evaporating dish and evaporated over a steam bath until the odor of alcohol was no longer perceptible. The remainder was then treated for removal of lipoid material which would interfere with the carbohydrate analysis. An excess of Horne's lead subacetate was added, the precipitate removed by filtering, and excess lead subacetate removed with sodium acid phosphate and filtering. The filtrate was then placed in a 500cc. volumetric flask and made up to volume. All material, when not being used, was kept in a refrigerator.

Total dextrins, starches, and hemicelluloses.

The residue of the original extraction was spread on an evaporating dish to dry off all alcohol.

The residue and original filter paper were placed in 400cc. of $2\frac{1}{2}\%$ sulphuric acid and boiled in a reflux condenser for $2\frac{1}{2}$ hours. After cooling, the solution was neutralized with sodium hydroxide and was ready for determination of its reducing power.

Determination of reducing sugars.

Reducing sugars in each case were determined by the Bertrand Method.

TABLE I

Carbohydrate Content of Seedlings of Different Ages and Grades*

Age & Grade	Weight in Grams: Dry Weight	Sugars	Poly-saccharides	Percentage of Total Dry Weight: Sugars	Poly-saccharides	Total Carbohydrates
4A	12.1567	1.4618	1.1184	12.02	9.20	21.22
B	4.7548	.7474	.4743	15.70	9.98	25.68
C	1.5142	.1472	.1321	9.72	8.72	18.44
3A	7.8083	.9034	.6108	11.57	7.84	19.41
B	3.0724	.4449	.3058	14.48	9.96	24.44
C	.9354	.0741	.1015	7.92	10.85	18.77
2A	2.4436	.1925	.2292	7.88	9.38	17.36
B	1.4538	.0762	.1442	5.24	9.92	15.16
C	.5070	.0297	.0516	5.85	10.11	15.96
1A	.4123	.0278	.0641	6.73	15.54	22.27
C	.2768	.0229	.0409	8.28	14.76	24.04

* Per-seedling data

TABLE II

Carbohydrate Content in Grams, Height Growth and Total Growth in Inches of Shoots of White Pine Seedlings of Different Ages and Grades

Age & Grade	Carbohy-drates. Grams	Rank	Height Growth Inches	Rank	Total Growth Inches	Rank
4A	2.5802	1	2.69	1	23.85	1
B	1.2217	3	1.76	6	7.43	3
C	.2793	6	.99	11	3.50	8
3A	1.5141	2	1.83	5	9.91	2
B	.7508	4	1.49	7	6.59	4
C	.1756	8	1.10	10	2.34	9
2A	.4217	5	1.90	4	5.91	5
B	.2204	7	2.16	3	5.86	6
C	.0812	10	1.20	9	2.28	10
1A	.0918	9	2.63	2	4.54	7
C	.0638	11	1.29	8	1.33	11

RESULTS

The growth habit of white pine during the first few years of its seedling stage is brought out by this experiment. During the first and second year there is usually a single stem which makes for rapid initial height growth--totaling, for the two years, from two to four inches. Beginning with the third year, however, extensive lateral branching and formation of from two to six leaders take place. This condition is intensified in the fourth year so that the seedling by the end of that year is rather low and bushy. The total amount of growth put on in the fourth year is greatly increased, but most of it is in the form of lateral shoots.

The growth actually obtained for the various ages and grades may have been influenced considerably by the fact that the natural trend of physiological activities was interrupted. The shock of transplanting was probably greater in the older seedlings than in the younger ones because a greater portion of roots was excised furing lifting operations.

Carbohydrates.

The sugar and polysaccharide contents of the white pine seedlings increase with age and quality of the stock. This is clearly brought out in Table I, page 11, and Fig. 1, page 19. In the one and two year seedlings, the weight of sugars is less than that of polysaccharides, but this standing is reversed in those three and four years old.

The percentage of sugars varies from 5.85 to 15.70, being high in the three and four year seedlings of A and B grade and low in the one and two year seedlings. Grade C stock of both three and four years shows a marked reduction in percentage of sugar as compared with the better quality classes. The one year seedlings have a slightly higher percentage of sugar than the two year seedlings.

The one year seedlings have the highest percentage of polysaccharides--close to 15 percent. In the other stock there is little variation in percentage of polysaccharides, averaging between 9 and 10 percent.

The percentage of total carbohydrates does not show an apparent correlation with age or quality of stock although it is lowest in the 2 year seedlings and highest in quality B seedlings of three and four years of age. It averages close to 20 percent for all seedlings. As the size of the seedling increases, the amount of available carbohydrates increase. The percentage of available carbohydrates in one to four year white pine seedlings is approximately the same regardless of any difference in age, grade, or size.

Although percentage of total carbohydrates is approximately the same for all ages and grades of seedlings, the component sugar and polysaccharide contents

are not in equal proportion. The percentage of sugars increases with age and increasing grade while percentage of polysaccharides decreases with age and, in most cases, is lower in the better quality stock. This may be expected because as sugars increase polysaccharides must decrease for many of them are broken down to mono- and di-saccharides which make up the sugars. Greater amounts of sugars are to be found in older age classes and better grades because there is a greater portion of living tissue in form of needles and apical meristems which constantly require sugar in their metabolism and growth. In one and two year seedlings there is usually a single leader and stem, while in the three and four year seedlings there are from two to six leaders and four to twelve lateral shoots with corresponding amounts of needles and cambium.

Height growth.

Height growth, which ranges from 1.0" to 2.7", is not closely correlated with either age or the carbohydrate content of the seedlings. Fig. 2, page 20, shows that there is a decrease in rate of height growth with increase in age from one through three years in both grades A and B, followed by a sharp rise in the fourth year. This decrease is continuous for the seedlings of grade C. First year height growth made by A and B grades was, on an average, more than twice that made by grade C stock. In grades A and B height growth was 2.1" while in grade C it was only 1.1".

With the exception of 2B stock, there is a decrease in height growth from grade A to B to C. This is clearly represented by Fig. 3, page 21, which also shows that the weight of total carbohydrates increases rapidly with age, while height growth, represented by a nearly horizontal line, remains almost uniform. Furthermore, a study of columns 3 and 5 of Table II shows that the order of rank of the height growth in inches does not correspond to that of weight of total carbohydrates in grams.

There is no significant correlation between total carbohydrates and height growth. This is brought out by the following statistical results:

*$M = 1.74$

$$s = \sqrt{Sx^2/N} = \sqrt{3.03/11} = .53$$

$$s_m = s/\sqrt{N} = .53/\sqrt{11} = \pm .16$$

Reliability of sampling and measuring is brought out by the fact that the mean of 1.74" will very within

* M -- mean
s -- standard deviation
s_m-- standard deviation of the mean or standard error
S -- summation
x --deviation of X from the mean
N -- number of observations

± .16" two out of three times if similar samples were selected at random.

$$*R = S(xy)/\sqrt{(Sx^2)(Sy^2)} = 2.348/\sqrt{21.820} = .502 \pm .249$$

A correlation coefficient of .502, when only eleven pairs of observations were considered, is non-significant. This strengthens the statement made previously that height growth is not closely correlated with the carbohydrate content of the seedlings.

The regression formula for determining height growth (X) when total carbohydrates (Y) are known is found as follows:

$$X = a + bY$$

$$= 1.49 + .37Y$$

Total growth.

Combined growth of all shoots on the other hand shows a very close correlation with total carbohydrates. The order of ranking of total growth in inches and total carbohydrates in columns 7 and 3, respectively, of Table II are similar. The curves in Fig. 4, page 22, representing the two variables, bring out very clearly the almost perfect correlation.

* R --correlation coefficient
y --deviation from Y
a --constant
b --constant

There is a highly significant correlation between total carbohydrates and total growth which is brought out by the following:

$M = 6.7$

$s = \sqrt{Sx^2/N} = \sqrt{390.82/11} = 5.96$

$s_m = s/\sqrt{N} = 5.96/\sqrt{11} = \pm 1.80$

$R = S(sy)/\sqrt{(Sx^2)(Sy^2)} = 47.09/\sqrt{2493.43} = .94 \pm .007$

The correlation coefficient of .94 gives assurance of a significant to highly significant correlation for all samples in spite of the fact that the mean of 6.7 has a standard error of 1.80.

The regression formula for determining the total growth (X) when total carbohydrates (Y) are known is:

$X = a + bY$

$= 1.85 + 7.20Y$

Total growth is very small for the younger trees, being only 4.5" for 1A seedlings and 5.9" for 2A. However it increases rapidly with age of seedling. The 3A stock, for instance, grew 9.9" while 4A stock had a total growth of 23.9". Suppressed stock of grade C made very little growth--1.3" in 1C and 3.5" in 4C seedlings.

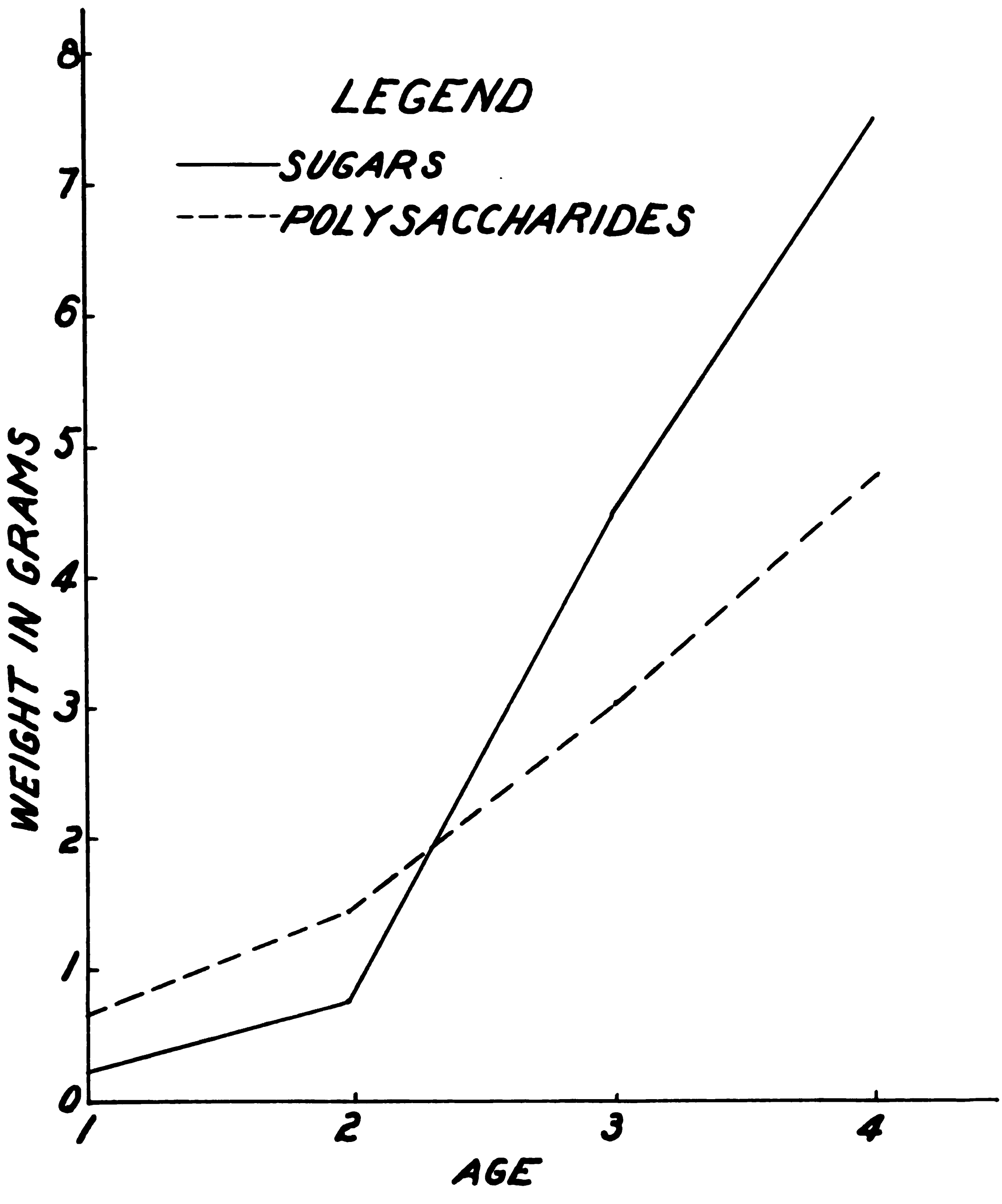

Fig. 1. Mean weight of sugars and polysaccharides per tree for grade B stock one to four years old.

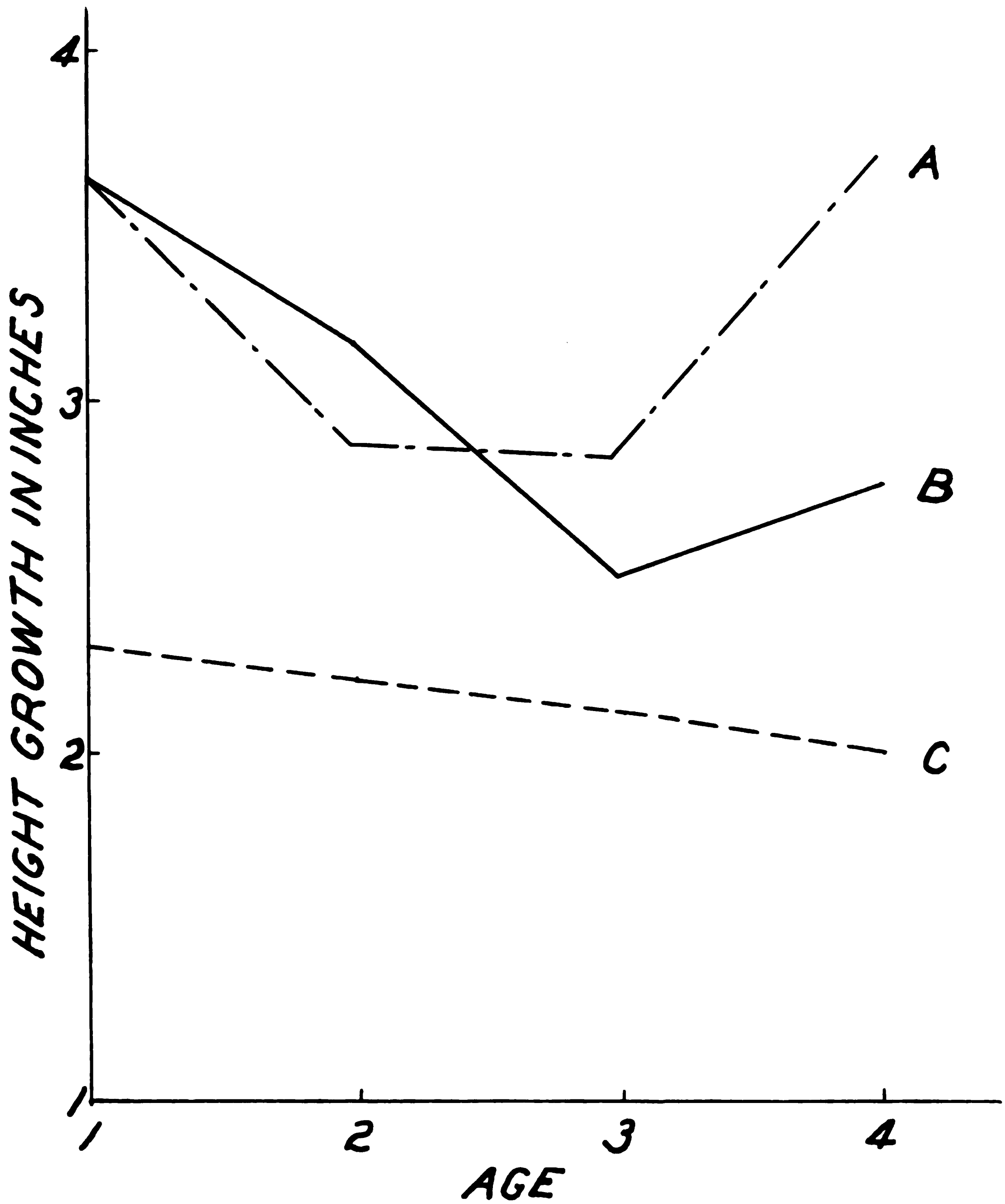

Fig. 2. Current annual height growth for stock of various grades.

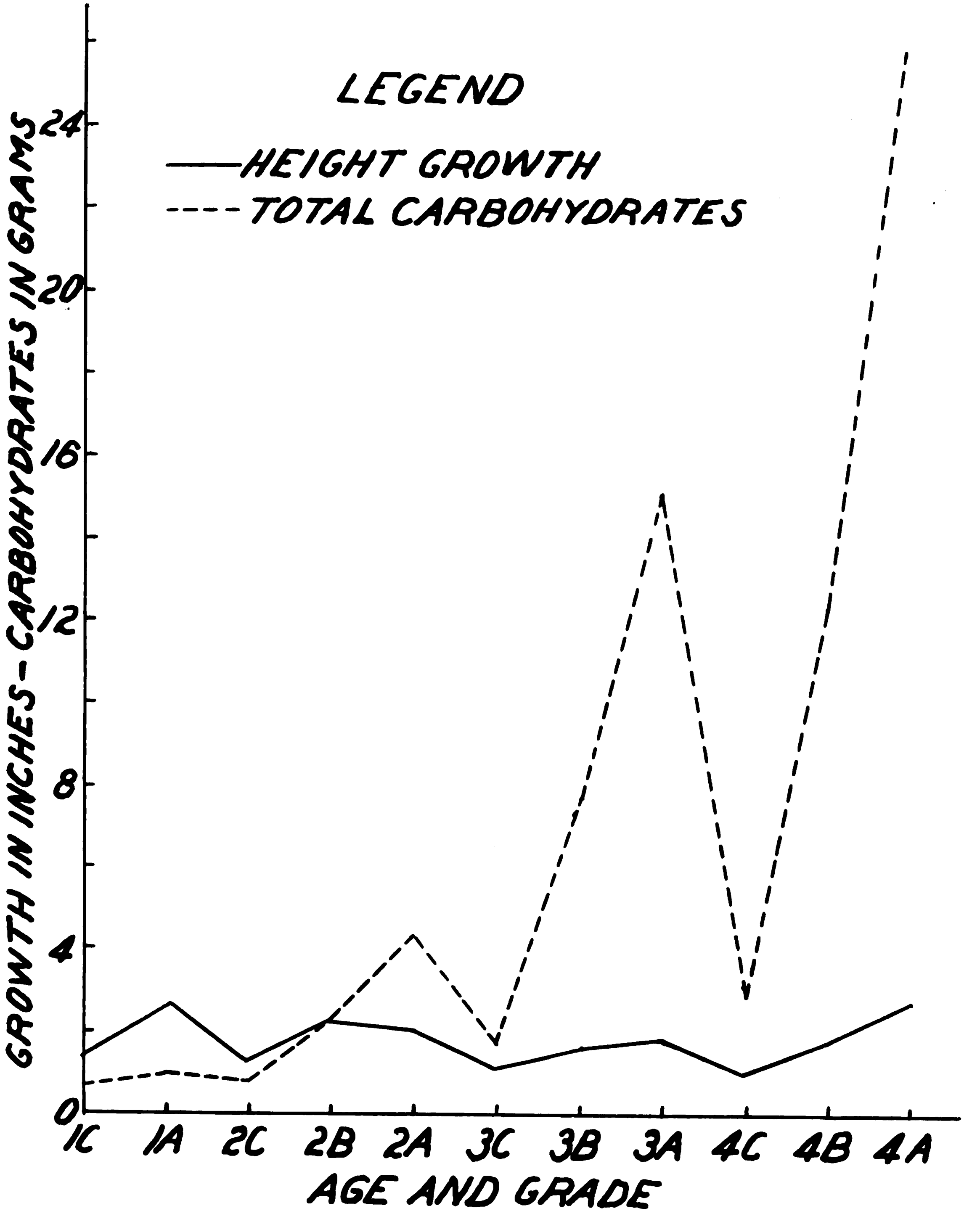

Fig. 3. Relation of height growth to total available carbohydrates.

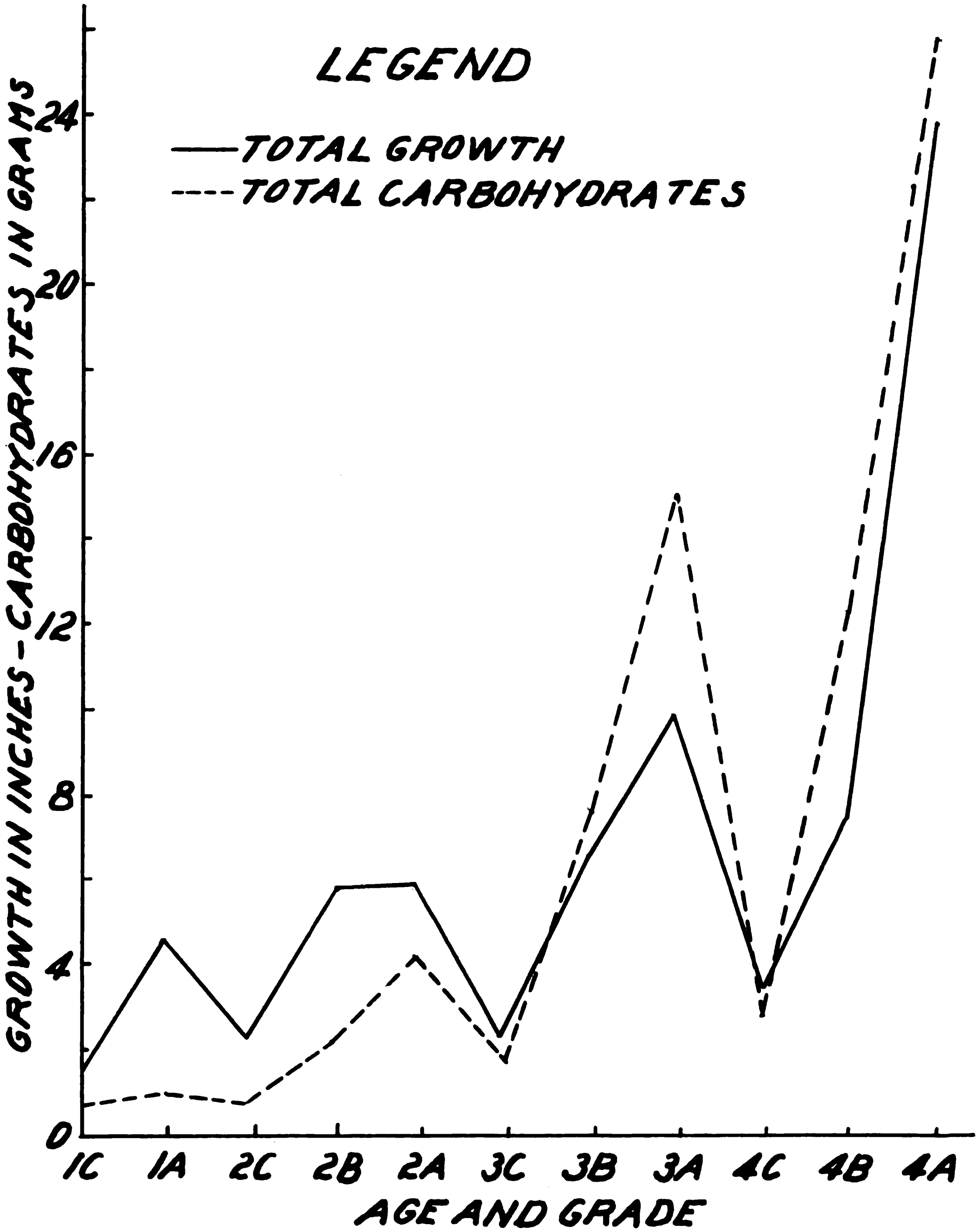

Fig. 4. Relation of total growth to total available carbohydrates.

SUMMARY AND CONCLUSIONS

A study was made of the carbohydrate content of white pine seedlings of four age classes and three quality classes in relation to height growth and total shoot growth.

The carbohydrate content of white pine seedlings increased with the age and the quality of seedlings. The dry weight of the seedlings likewise increased with age and quality of stock.

The percentage of carbohydrates in white pine seedlings one to four years old and of three quality classes appeared to be independent of age, quality or size of seedling.

Percentages of sugar increase with age and quality of seedling.

Percentages of polysaccharides show little correlation with age and quality of white pine seedlings. They are highest in one year seedlings.

Total available carbohydrates were not correlated significantly with height growth of the seedlings. Lack of significant correlation may be explained by the fact that white pine seedlings often do not have a well developed terminal shoot and little or no height growth may be made by seedlings in some years. The correlation coefficient for available carbohydrate content and

height growth was .502 $\pm$.249 with eleven series of measurements. This is not significant.

A high correlation was determined for available carbohydrate content and total amount of shoot growth. A correlation coefficient of .94 $\pm$.007 was obtained with eleven series of measurements. This is highly significant. For each gram of available carbohydrate there is very close to one inch of shoot growth.

Carbohydrate content of white pine seedlings therefore serves as an excellent criterion for the prediction of growth in white pine seedlings.

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