SOME EFFECTS OF DIFFERENT FERTILIZER TREATMENTS ON THE DIURNAL AND SEASONAL CHANGES IN THE SUGAR CONTENT OF THE SAP AND TISSUE OF POTATO PLANTS

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INTRODUCTION

Because of the large increases in yields resulting from the application of fertilizer salts to fields that ordinarily produce plants which from all appearances are normal and vigorous, much interest has been shown in studies of the growth habits, nutrient requirements, and physiological processes of plants.

The problems involved in the search for a more intimate understanding of the way plants respond to stimulations of any kind are many and have attracted workers from many fields of endeavor. Much work has been done on external factors such as climate and the physical and chemical nature of the soil. Studies of the plants themselves considering their anatomy, ecology, habits, method of reproduction and chemical analyses of the various parts of plants have been made and from all of this work much valuable information has been obtained. Among the many chemical analyses made of plant tissues, carbohydrate analysis of various parts of plants and the study of the rates of movement of these constituents is one of the most interesting phases of all chemical analyses. The study of carbohydrate variations is a difficult one because of the large number of carbohydrates and the wide range of fluctuations in concentrations from hour to hour. Most of the work done in the past has been directed towards an attempt to determine how carbon assimilation takes place, and how the products of carbon assimilation are transported from the leaves to the parts of the plant in which they are utilized or stored for future use. Although some studies have been made showing the tendencies of different carbohydrates to vary in concentration in different parts of plants throughout the day, the need for a more complete knowledge of these changes, and how the effect of certain cultural conditions affect the normal growth of plants was felt.

This work was planned so as to find out more about the variation in the carbohydrate concentrations resulting from applications of fertilizer salts in amounts that have proven practical in increasing the yields of potatoes. The potato plant was selected because the author had been working with fertilizer requirements for potatoes for several years and also, because of the habits of the plant in storing carbohydrates, it lends itself very well to this type of investigation.

HISTORICAL

Sachs[1] in 1862 first proved that the production of starch in the chlorophyll granule depends upon the action of light and that the starch formed during the hours of sunlight is wholly or partially redissolved from the leaf during the night to supply the demands of the growing points of plants. This discovery by Sachs aroused great interest in studies of carbon assimilation.

Probably the first theory on the problem of carbon assimilation was put forth by Liebig [2] in 1843, in which he considered that organic acids were the intermediate products. This theory was not based upon experimental evidence and found little or no support. A theory that has directly influenced so much experimental work is the formaldehyde theory, in which it is claimed that formaldehyde is formed from water and carbon dioxide in the green part of the leaf. This formaldehyde is immediately condensed to sugar. The formaldehyde theory was first proposed by A. Baeyer (3) in 1870 and is widely accepted at the present time. The first sugar formed according to this theory is grape sugar, d - glucose, which is later enolized to fruit sugar - fructose and from glucose and fructose, sucrose is formed. From sucrose and the simpler sugars the more complex carbohydrates of storage such as starches, hemicelluloses and celluloses are formed.

Most of the early work along this line of investigation consisted of attempts to prove this theory. The methods used, however, were almost entirely qualitative and as such are now subject to much criticism.

In 1893 Brown and Morris (4) who were among the first to use quantitative methods in these studies came to the conclusion that sucrose was the primary sugar of photosynthesis. They found sucrose to be much more abundant in the leaf than starch and the method in which it fluctuated caused them to draw this conclusion. They considered dextrose and fructose to be the products of hydrolysis of sucrose rather than those materials from which it was synthesized.

Parkin (5) working with the snowdrop, found that at different periods during the day even when the leaves of the plant were covered with black paper, the concentration of the hexoses remained about constant while the concentration of sucrose varied with the amount of sunlight and temperature of the day. He concluded from this that sucrose was the primary sugar of photosynthesis.

Davis, Daish & Sawyer [6] working with translocation of carbohydrates in the leaves and stems of the mangold, and Davis & Sawyer [7] working with translocation of carbohydrates in the potato plant, found that during early stages of growth the content of sucrose was always greater than that of the hexose in the leaf, but the reverse was true in the stems. They claimed this to be proof that in the leaf sucrose is the primary sugar of photosynthesis and is converted into the hexoses for means of transportation. Their reports contain an excellent review of the literature on the subject of the primary sugars of photosynthesis up to their time.

Dixon & Mason [8] made microchemical examinations of the assimulating cells of a number of plants and found that there was a considerable concentration of hexoses in the chloroplasts or in the protoplasm immediately surrounding them. Sucrose on the other hand was concentrated in the vacuoles and invertase was held apart from it in the protoplasm. They concluded from this that the hexoses are first formed from formaldehyde in the chloroplast and where their concentration reaches a certain limit condensation into sucrose due to invertase or some other saccharigenic enzyme takes place.

Priestly [9] took the rather unusual view that sucrose is not a product of photosynthesis, but a decomposition product of protoplasm. He based this view principally upon the fact that chemically sucrose had never been synthesized from fructose and dextrose, and no enzyme had been found in the plant to which could be ascribed this function. In his paper he gives an excellent review of the literature or the subject especially with reference to papers concluding the hexoses, especially dextrose to be the primary sugar of photosynthesis.

Clements[10] in making carbohydrate studies of the leaves of several plants concluded that dextrose is the primary sugar of photosynthesis primarily because of the way it varies in concentration in plants in comparison to the variations of sucrose.

The subject of carbohydrate - nitrogen ratio in plant tissue has been of great interest to horticulturists. A consideration of the purpose for growing the plant has been of prime importance in this field. In cases where the purpose is to produce vegetative growth, a different carbohydrate nitrogen ratio is required than when the plant is grown primarily for reproductive growth.

Among the outstanding researches in this line is that of Kraus & Kraybill[11]. Working with the tomato plant they found:

1. Though there be present an abundance of moisture and mineral nutrients including nitrates, yet without the available carbohydrate supply, vegetation is weakened and plants are not fruitful.
2. An abundance of moisture and mineral nutrients, especially nitrates, coupled with an available carbohydrate supply makes for increased vegetation, barrenness and sterility.
3. A relative decrease in the nitrates in proportion to the carbohydrates makes for an accumulation of the latter, and also for fruitfulness, fertility and lessened vegetation.
4. A further reduction of nitrates without inhibiting a possible increase in carbohydrates, makes for a suppression both of vegetation and fruitfulness.

Reid [12] working with basal and upper cuttings of tomato plants found those high in nitrogen furnished favorable conditions for shoot growth while those high in carbohydrates appeared to furnish better conditions for root growth.

Hooker[13] working on the changes in chemical composition of apple spurs, Murneek[14] on the nitrogen and carbohydrate relations in (organs of) apple bearing spurs, Harvey & Murneek[15] on the carbohydrate and nitrogen relations in apple spurs all show that the carbohydrate and nitrogen ratio bears an important part in determining whether or not a spur will be fruitful or barren. These workers all came to the same conclusions as Kraus & Kraybill.

A very noteworthy piece of work on carbohydrate studies was done by Mason and Maskell[16] in studying the translocation of carbohydrates in the leaf, stem, bark and boll of cotton plants. They were able to assertain the rate of movements in specialized parts of the plant. They also found that there was a lag in the cycle of maximum carbohydrates from leaves to bark.

Miller[17] working with the leaves of corn and sorghams found that in most cases the non-reducing sugars were in excess of the reducing sugars. The non-reducing sugars increased markedly during the day and decreased during the night while the reducing sugars as a rule showed very little increase and the amounts present at the different periods of the day were irregular.

Janssen & Bartholemeu[18] found in working with tomatoes grown in sand cultures that there seemed to be an optimum potassium concentration which was condusive to the normal assimilation of carbohydrate compounds, and above or below which assimilation was reduced. This optimum relation was not found.

The same investigators[19] also found by working with a variety of field crops both on sand and water cultures, that maximum concentration of carbohydrates were produced at concentrations of 2 to 3 ppm of potassium, while applications of from 50 to 450 pounds of potassium chloride per acre to the soil on which the same crops were grown showed no correlation between the amount of potassium added to the soil and the carbohydrate content of the plants. They also found reciprocal relations between the potassium and nitrogen content of the plants when grown on sand or water cultures, but not with those grown in the field.

Clements[20] found reciprocal relations for nitrogen and carbohydrates in water cultures using field peas. However, he found the highest percentages of carbohydrates in the cultures that received the most calcium nitrate and the highest percentages of nitrogen in the cultures receiving high proportions of potassium di-hydrogen phosphate.

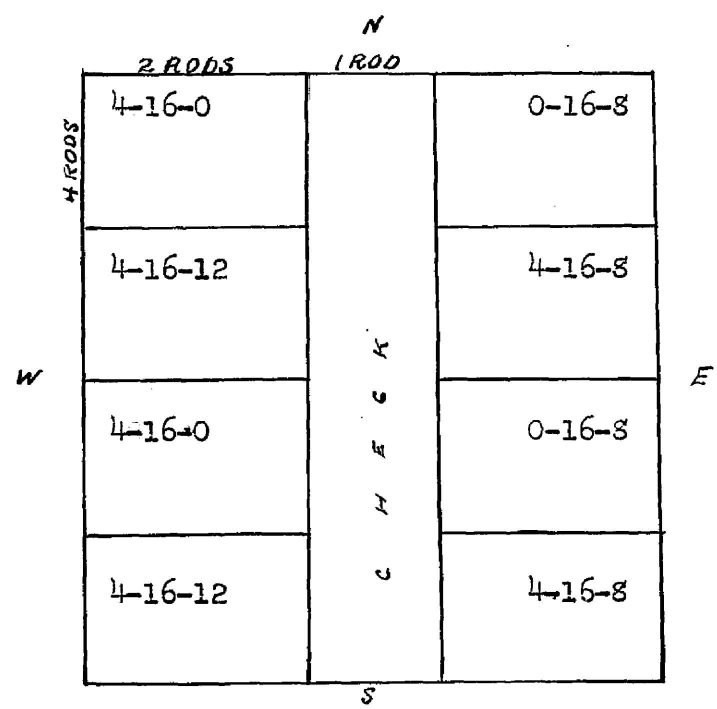
Woo[21] working with Amaranthus Retroflexus, a plant that is capable of storing large quantities of nitrogen found there was a reciprocal relation between carbohydrates and nitrogen, both in the leaves and the stems. This reciprocal relation was especially noticeable between insoluble protein and carbohydrates doubtless, because the carbohydrates are utilized in the formation of proteins.

Hartwell[22] found that a deficiency of available potassium in the soil was usually accompanied by an accumulation of starch in potato vines. He also found that many different factors which correlated in each case with retarded growth, were found to be associated with an accumulation of starch in the above ground portion of plants.

Experimental Outline.

The object of this experiment was to study the diurnal and seasonal variations in the sugar content of both leaves and stalks of the potato plant, for the purpose of noting any differences that might occur due to the application of commercial fertilizers under field conditions, and to see if these differences could be correlated with yields.

The diagram below gives an outline of the way the field from which the samples were gathered was laid out.



The treatments were replicated four times, the other two replications joining the above on the south. The samples were taken from the check plot, the 4-16-0, 0-16-8 and 4-16-8 of the north series and the other three series were left for taking yields. The 4-16-12 plot was not sampled because previous experiments had shown that there was no difference in yields from plots fertilized with 4-16-12 and 4-16-8, and it was considered advisable to keep the number of samples as low as possible. The 4-16-12 treatment was included in order to further compare it with 4-16-8 from a standpoint of increasing the yields.

The field used for this experiment was located about a mile and a half from the laboratory. The soil was Hillsdale sandy loam, which is one of the principle morranic soils of south central Michigan and is well adopted to the growing of potatoes. The fertilizers applied were made from $(NH_4)_2SO_4$ as the nitrogen carrier 40% superphosphate as the phosphorous carrier and muriate of potash as the potassium carrier. It was mixed and broadcast by hand at the rate of 750 pounds per acre. The fertilizer was worked into the soil with a spike-tooth drag before the potatoes were planted.

Weather Conditions

The season of 1930 was an extremely dry one, the yields of potatoes being consequently greatly reduced. The yields obtained showed no differences due to treatment and only ~~yielded~~ amounted to from 90 to 100 bushels per acre while in years of normal rainfall from 200 to 300 bushels per acre could be expected.

The plots were sampled on July 23, August 12 and September 4, and over this period only 0.58 of an inch of rain fell while the normal for this period is from four to five inches. The first samples were taken when the blossoms were forming, the second as the plants began to set tubers, and the third sampling when the plants began to mature.

An attempt was made to take samples only on a clear day, and samplings were never taken unless the day previous had been clear. This precaution was taken in order that the normal production and utilization of carbohydrates might be going on within the plant at the time when the samples were taken. The following data from the United States Weather Bureau at East Lansing give the meteorological conditions on the dates of sampling and for two days previous to each sampling.

Date	Min. Temp.	Max. Temp.	Character of Day	Sunshine per cent	Precipitation in inches
July 21	67	89	pt. cloudy	68	.04
22	63	82	" "	72	0
23	58	83	clear	99	0
August 10	52	73	clear	96	0
11	45	73	"	94	0
12	45	73	"	100	0
September 2	68	87	cloudy	23	.07
3	54	82	clear	95	0
4	46	79	"	100	0

In spite of the dry weather the plants seemed to be perfectly normal and unhurt during the first two samplings. The plants showed that the drought was hurting them by the time the third sampling was made. They were fresh during the night, but during the day the top leaves curled slightly and the plants looked wilted. When the first samples were taken the plants in the fertilized plots were much larger than those in the check rows. This difference was not so noticeable when the second samples were taken showing that the larger plants were not growing as fast as the smaller ones. Even in normal years much greater differences between fertilized plots are noticed in the earlier part of the season than can be detected later on. By September 4 when the third samples were taken the plants showed clearly that they were injured by the prolonged dry spell. The leaves curled up and the plants looked slightly wilted from the middle of the day on until after dark, although they looked fresh in the morning. No differences could be seen in size between the plants in the check rows and on the fertilized plots.

Methods of Sampling.

At each sampling seven sets of samples were taken. Starting at midnight samples were taken every four hours through midnight of the following day. In taking samples the whole hill was selected, tops being cut off about two inches above the ground. Sufficient hills were taken to fill a twenty pound capacity paper bag. In the first sampling six hills were taken from each fertilized plot, but eight hills were required from the check plot to give sufficient material. It was only necessary to take four hills from each fertilized plot and five from the check plot for the second sampling whereas four hills gave sufficient material for each from all treatments in the third sampling.

Preparation of Samples.

The samples thus obtained were rushed to the laboratory immediately after cutting and disposed of as quickly as possible. The cutting from each treatment was separated into leaves and stalks. Part of each was then chopped up as fine as could be conveniently cut with a paper cutter and 50 gms of each was put in separate pint jars containing 400 cc of boiling alcohol to which had been added a little calcium carbonate to neutralize any acid that might be found in the plant. The use of the paper cutter permitted the leaves to be cut in strips about 1/4 of an inch wide while the stalks were cut in sections about half an inch in length. The jars were set on the steam bath to boil for one hour in order to kill the enzymes immediately after the tissue had been added to them. The remainder of the sample of leaves and stalks was ground separately in a food chopper and then from 100 grams of each the juice was pressed out according to the method described by Sayre and Morris[23]. Never did the amount of juice pressed out from the leaves exceed 50 cc. It was measured and all of it poured into bottles containing 200 cc of boiling alcohol which had a little calcium carbonate added. The juice from 100 grams of ground up stalks usually amounted to from 60 to 70 cc, 50 cc of which was pipetted off and preserved in the same manner as the sap from the leaves. The samples thus obtained were also put on the steam bath and boiled for one hour. All of the samples from both tissue and sap were tightly sealed after heating for one hour and stored away to be analyzed later.

In order to have the samples comparable in all respects, the same order of handling was maintained in the laboratory for all samples so that the time between cutting in the field and the killing of the enzymes would be about the same for each treatment throughout the period of sampling. The time between cutting the samples in the field and getting all samples into boiling alcohol was just about one hour. Although it would have been better to have had less time between cutting in the field and getting them in hot alcohol, there were so many samples that they could not be carefully handled in any shorter time.

Samples of both sap and tissue were preserved because it was considered possible that additional information might be gained by an analysis of the sap.

An attempt was made to determine the sugar content of sap without the use of preserving agents. In this attempt, the diluted juice was treated with neutral lead acetate to clearify the solution. The precipitate caused by the lead acetate was thrown down by centrifuging and the clear liquid poured off into another centrifuge tube. The lead was precipitated from solution by using powdered sodium oxalate and the precipitate removed by centrifuging. The supernatant liquid was poured off into a dry container and an aliquot taken made up to volume ready for analysis. The solutions prepared in this manner and determination made immediately had about the same reducing power as samples that were preserved in alcohol. Their reducing power did not remain constant and at the end of three or four days they exhibited very little if any reducing power. As it was impossible to make the determinations immediately after sampling it was necessary to find some way of maintaining the reducing power of the samples constant. The method of preserving in alcohol was known to be satisfactory, but before determinations could be made, it was necessary to change the samples from an alcohol solution to a water solution and a search was made for a more convenient method.

Ripperton[24] working with carbohydrate metabolism in the edible canna found that when he took the expressed sap and clearified it with lead and then removed the lead and made up to volume the reducing power of these samples changed rapidly, and he could not use this method. He next tried using formaldehyde and found that by adding formaldehyde, the percent of total sugars remainded constant, but the sucrose continued to invert. He found the method of preserving in alcohol quite satisfactory.

Further searches for a preservative so as not to necessitate the use of alcohol showed that the use of a half gram of sodium fluoride maintained the reducing power of samples constant for at least ten days. Other samples in which the lead acetate was used as a preservative and was not removed until just previous to making the determination proved effective in maintaining a constant reducing power in the solutions. It is usually considered that the presence of lead in fructose solutions will decompose the fructose and thus change the reducing power. These solutions treated with lead acetate and left to stand did not change their reducing power as they must have been rather low in fructose.

Although the use of either sodium fluoride or lead acetate indicated a possible method of preserving samples, before any further investigations could be made it was necessary to sample the field, and hence both the sap and the tissues were preserved in alcohol.

Methods of Analyses.

The samples that were preserved in alcohol were filtered and made up to volume. From these, aliquots were taken and the alcohol removed by distillation under reduced pressure using Classion flasks. The solutions in the Classion flasks were boiled down to about 30cc, then washed out into 100 cc volumetric flasks with hot water. The volumetric flasks were then cooled down rapidly by setting them in running tap water for several minutes.

then allowed to stand until cooled to room temperature. At this time they were made up to volume, shaken well and the contents poured into 100 cc centrifuge tubes, which previously had about one tenth of a gram neutral lead acetate added. The solutions were allowed to stand for fifteen or twenty minutes to permit all of the colloidal material to be precipitated. The precipitate formed was thrown to the bottom of the tube into a sticky mass by centrifuging. The supernatant liquid was poured off into another dry centrifuge tube and powdered sodium oxalate added to precipitate all the lead. A second centrifuging threw down the lead oxalate precipitate and the supernatant liquid was decanted into a dry beaker. An aliquot of this liquid was made up to 100 cc volume.

The samples thus prepared were thoroughly shaken, 50 cc of the solution put in a 300 cc Erlenmeyer flask for reducing sugar determination and the remaining 50 cc put into another 300 cc flask to which had been added 5 grams of citric acid crystals used to hydrolyze all sucrose. The samples for sucrose analysis were next taken, covered with 100 cc beakers and put on a gas hot plate and permitted to boil for ten to fifteen minutes to hydrolyze the sucrose. The citric acid was next neutralized with concentrated sodium hydroxide to a pink color with phenolphthalein.

The reducing power of both the sucrose samples and non-reducing sugars was determined by the Shaffer and Hartman[25] method, using a standard solution of sodium thio-sulphate to titrate the excess iodine from a known amount of potassium iodide-iodate added to the reduced copper solutions. Twelve cc of 8N sulphuric acid were immediately added to the solution after the iodide-iodate had been added, shaken for a few seconds until all the cuprous oxides had been dissolved, then 20 cc of saturated potassium oxalate was added and the solution titrated with standard sodium thio-sulphate solution. As the end point of the titration was nearly reached, 5 cc of starch solution was introduced as an indicator and the titration completed.

Due to the buffer effect of the citrate ion in the sucrose samples it was necessary to add an additional 5 cc of the sulphuric acid in the titration to bring the samples to the necessary pH.

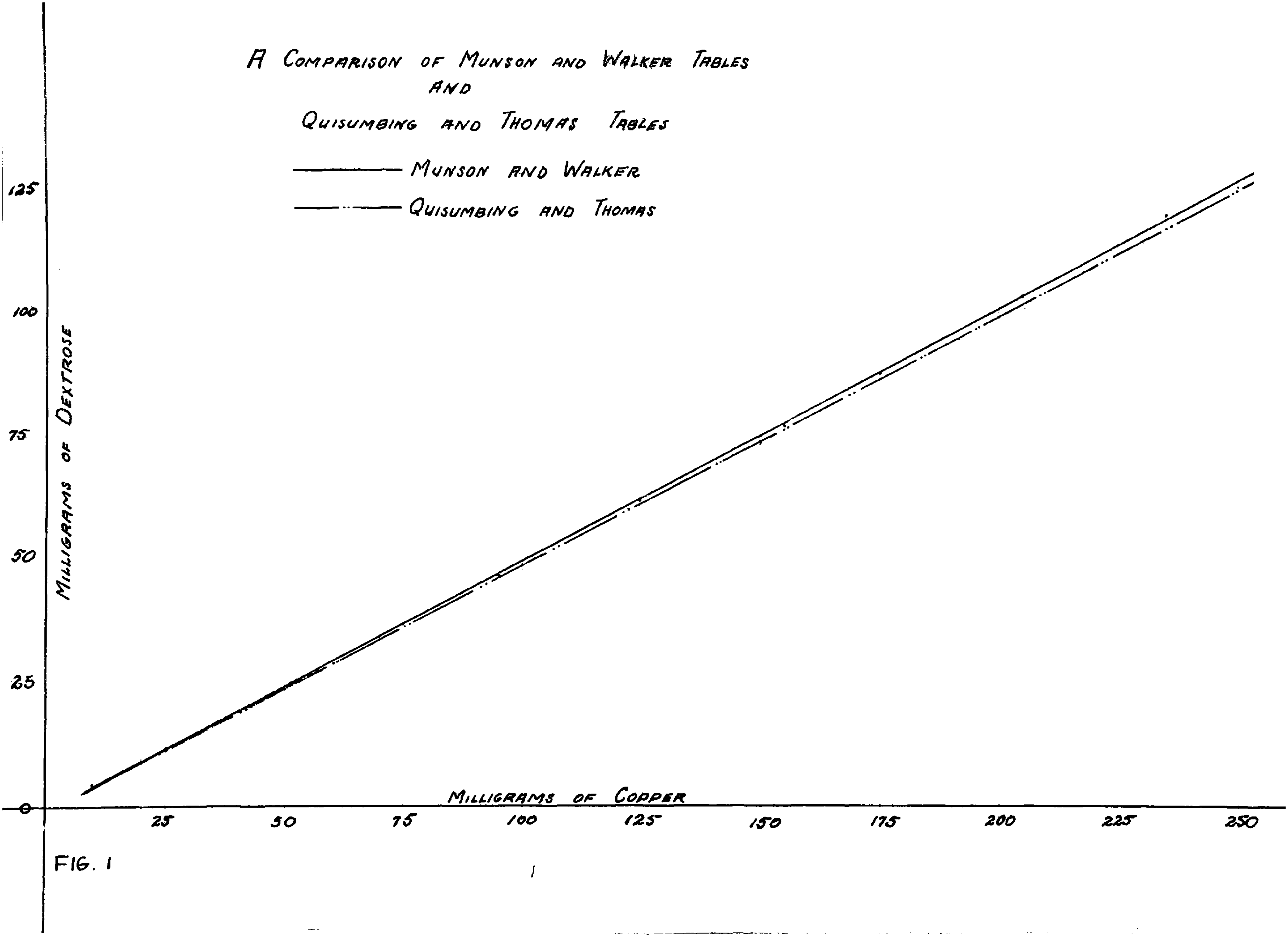
The committee on methods for the society of plant physiology[26] object to the use of the Shaffer and Hartman methods on the ground that for various tissues it is not always possible to get a sharp end point in the final titration. In this work, by using the recommended 15 cc of 5 normal sulphuric acid the end point was not always sharp, but by increasing the strength of the acid a clearly defined end point could always be obtained. It appeared, at least, with the tissue used in this work, that there is a rather narrow range of pH in which a sharp end point can be obtained with this titration. If an excess of acid is added a white precipitate forms in the solution and the resulting titration values are too low, while if the pH is too high, the end point is indistinct and the results are unreliable. The addition of extra acid, in the case of sucrose hydrolyzed by citric acid, to correct for the buffer effect of the citrate ion indicates the necessity of getting a proper degree of acidity in the solutions before a clear end point can be observed in the titration. With a little practice one can usually tell by the color of the solutions as the acid is being added, just how much acid to use and if working with tissue from only one kind of plant, the correct acidity can soon be found and no further difficulty will be noted.

If the difficulty lies in obtaining a definite pH value before titrating, it is easy to understand why all tissue cannot be handled alike because it is easy to conceive of different tissues exerting different buffer effect on solutions, which would necessarily have to be corrected for before sharp end points could be obtained.

There are a number of other methods of determining the amount of reduced copper (26, 27), but the Shaffer & Hartman method has a distinct advantage over most of them in that it is not necessary in this method to filter off the products of the reaction before the titration can be made for the determination of the reduced copper. An excellent review of the literature on methods is given in the former reference.

The method of reducing the copper described by Quesumbing & Thomas (28), was employed in this work because it was more convenient in handling a number of samples at one time than is the Munson & Walker[(29)] method. Although the Quesumbing & Thomas method of reduction was used, the Munson & Walker tables were used for calculating the amount of sugar from the amount of copper reduced, because the Munson & Walker tables are so much more complete. The curves on Figure I show the variations between the two tables within the range of copper obtained in this work. The difference between the two is so slight that the error due to interpolation of the smaller tables of Quesumbing and Thomas would be greater in many cases than the difference between the two tables.

In working out the method of analysis it was desirable to work out a plan whereby a set of eight samples, which is the number of samples that were taken at each cutting could be handled in a day. This was desirable in order to have the method of handling for all samples as nearly alike


A Comparison of Munson and Walker Tables
and
Quisumbing and Thomas Tables
Munson and Walker
Quisumbing and Thomas
Milligrams of Dextrose
Milligrams of Copper
0
25
50
75
100
125
150
175
200
225
250
FIG. 1

as possible even to the time between starting a determination and the final titration. In adopting such a method it was necessary to choose certain procedures, such as distillation under reduced pressure rather than evaporation from a steam bath to get rid of the alcohol, and the use of citric acid hydrolysis for sucrose rather than inversion by invertase, because these proceedures were a little faster and fitted in better with the scheme of analysis. The use of the centrifuge in removing precipitates from leading and deleading was much more rapid and convenient than filtering for this purpose. In using any more rapid method, a careful check up was always made in order to see that accuracy was not sacrificed by the use of a more rapid method.

Discussions and Conclusions

The data are presented in the form of graphs, which seems to be the most effective way of showing diurnal variations. All of the free reducing sugars are reported as glucose and all material hydrolyzed with ten per cent citric acid solution is reported as sucrose. Series A, figures 2-7 shows the diurnal variations in the carbohydrate content of all the samples, due to the effect of the different fertilizer treatments. All figures are given as percentage of green weight.

The curves in figures 2-7 follow almost the same course, showing that if there is any variation due to the different fertilizer treatments the variations are indeed small. Some irregularities are seen in some of the curves, but a careful observation shows that these irregularities are not consistant with any one treatment, but all of which show some irregularities. The data show that under the conditions of this experiment no differences due to differences in fertilizer treatment can be expected. This is consistant with the work of Janssen and Bartholemeu[19] who worked with a number of field crops, in the field and in sand and water cultures. In growing the crops in the field they applied constant amounts of nitrogen and phosphorus, and potassium varying in amounts from nothing up to 450 pounds of potassium chloride per acre. In this case no variation in the carbohydrate content of the tissue due to different amounts of potash added was observed. When the same plants were grown in water cultures and sand cultures they found a maximum carbohydrate production in solutions containing from two to three parts per million of potassium.

Clements[20] found in water cultures that a high carbohydrate content in the tissues of field peas was correlated with a low nitrogen content. The point of maximum carbohydrate production in the works of both Clements and Janssen and Bartholemeu are not always the points of maximum production or maximum growth.

Kraus & Kraybill[11] working with sand cultures found definite relations between nitrogen and carbohydrate content in the tissue of tomato plants. They were able to establish from these relationships some fact pertaining to the functions of different parts of the plant with reference to vegetation or reproduction.

It seems that results obtained with sand and water cultures under conditions in which the concentrations of the solutions in contact with the roots of plants are kept constant cannot be easily duplicated in field plot work. The difference in all probability rests in the fact that soil conditions are dynamic and not under control. There are wide variations in moisture supply and an extreme variability in the soil solution in the field. Both the per cent of moisture and the concentration of the soil solution are affected by so many factors under field conditions. Every rain dilutes the soil solution, the losses of moisture by transpiration tends to concentrate it while the thermal movement of water in the soil has its effect on the soil solution. Lyon & Buckman[30] give a good description of the dynamic character of the soil solution and its relation to the moisture contents of the soil. Factors of this nature appear to exert so much more influence on the plants that the effect of added fertilizer salts although sufficient to cause marked influences in yields seldom show any influence on the concentrations of carbohydrates in plants. This suggests that it may be possible by using water culture or sand cultures to adjust differences of concentrations of different nutrient elements between narrow enough limits so that distinct differences in growth of the plants may be observed without any noticeable differences in the carbohydrate content of the tissues or sap of the plant.

A further examination of the curves in series A indicates closer relationship between the curves of figures 2 and 3 than those of figures 4-7. The first mentioned curves are those from the first sampling on July 23, when there was not as much sugar found in the plants

as there was later. It is only natural to expect then, that when there is a larger amount of sugar in the plant any variations that may occur due to various factors, will cause greater differences than will occur under the same conditions in plants that have a much lower sugar content.

The curves in both the second and third samplings show greater irregularities in data from the stalks than in that from the leaves when both tissue and sap analyses are considered. There seems to be no apparent reason for this variation. An observation of the alcohol extracts indicates from a prior reasoning that the data from the leaves would be more irregular because of the larger amounts of coloring matter and the greater difficulty in clarifying the solutions.

With the exception of set (a) at the top of figure three, all of the curves in figures two and three are almost horizontal. The curves for glucose in the second and third samplings, figures 4-7, are horizontal or nearly so, while those for the sucrose seem to indicate clearly a minimum from 4 A.M. to 8 A.M. and a maximum between noon and 4 P.M. In the second sampling a minimum occurs at 8 A.M., but in the third sampling the minimum may be either at 4 A.M. or at 8 A.M.

A comparison of the amounts of hexoses reported as glucose, and of sucrose (figures 7-13) shows that in all three samplings the amount of the former is in excess of the latter in both the leaf and stalk samples. These findings are directly opposite to the findings of Davis and Sawyer(7), who found that samples taken on July 16 and 17, 1914, showed sucrose to be greatly in excess of hexoses at this time. They did find, however, that the percentages of hexoses seemed to remain constant throughout the day and night whereas the sucrose content showed a steady rise from 6 A.M. until about 2 P.M., at which time there was a gradual decrease until the minimum was reached at 4 A.M. In the stalks of the same sampling they found the hexoses to be greatly in excess of the sucrose, the curves for the hexoses and sucrose running almost parallel and showing a slow but steady rise from 6 A.M. until sunset and then dropping off to a minimum about 2 hours before sunrise.

Although the general trend of the curves for both sucrose and glucose in this study are similar to those of Davis and Sawyer, the amounts of sucrose as compared to glucose in the leaves are directly opposite. Miller[17], working on the carbohydrate variations in corn and sorghum leaves found in practically all cases that the percentages of non-reducing sugars were much greater than those of the reducing sugars in both types of plants.

Clements[10] results, on sunflowers, potatoes and soy beans do not conform with those of Davis & Sawyer or Miller. He found on July 6 and 7 that in the leaves of potatoes the curves of both simple sugars and sucrose are almost horizontal and the amounts of simple sugars are greatly in excess of sucrose. The curves for both sunflowers and soy beans on the same dates show the curves for both simple sugars and sucrose also to be about horizontal, but for these plants the amounts of sucrose are almost equal to those of glucose. Samples of potato leaves taken August 11 and 12, and 26 and 27 also show the amount of simple sugars to be in excess of sucrose, although, the percentages of both have increased over those from the July 6 and 7 samplings. In both of the later samplings the curves for the simple sugars are extremely irregular. This is also true for the later samplings of sunflowers and soy beans. There seems to be no definite cycle similar to that noted in the work of Davis and Sawyer and Miller, and also in the second and third samplings of this present work.

It seems logical to expect a rather definite cycle showing periods of maximum and minimum concentrations in sugars throughout the day. The work of Parkin, on the carbohydrate in the snowdrop and Mason & Maskell[16] on transport studies of carbohydrate in the cotton plants also seem to point out that there is a definite cycle in the sugar content of leaves of plants. In the work of Mason & Maskel and that of Parkin [5] samples were taken at six hour intervals, in this investigation at four hour intervals, and in that of Davis & Sawyer and of Miller at two hour intervals. Clements took his samples at intervals of one hour. Due to the great irregularities in his curves Clements concluded that intervals of one hour were too great so he sampled sunflowers on September 15, 1926, at intervals of 10 minutes between the hours of 11 A.M. and 2 P.M. In this case his curves were much smoother, but still there were rather large variations in the concentration of simple sugars between the 10 minute intervals.

A comparison of the percentages of sugars between the sap and tissue of the same samples (figures 8 to 13) shows that although the curves do not lie as close together as might be expected, yet the general trend of the curves are the same. The irregularities in the curves are not consistent and no factor of conversion can be obtained to correct for the difference between the two. The variations in the first sampling seem to be just as wide as those in the second and third samplings.

In a number of cases the comparison curves run just as close together as those of Sayre and Morris [23] who made studies of the sugar content in the blades, sheaths and stems of corn.

The third set of graphs, Series C, figures 14 to 22, compare the amounts of the different sugars in the samples at different times of sampling. As might be expected, the concentration seems to be generally higher the later the sampling, at least the range of concentration is greater for the older plants. The later samplings also show a much greater irregularity in concentrations than is shown in the first samplings. There seem to be no variations due to different fertilizer treatments that are brought out by this manner of arranging the curves.

A careful examination of the curves presented in this work as well as those presented by others who have worked on diurnal variations in carbohydrate concentrations in plants, reveals a great fluctuation in all forms of carbohydrates studied. These fluctuations vary to a greater extent in older plants than in the younger ones. The curves for the later samples of sunflowers, potatoes and soy beans, as shown by Clements[10] are extremely irregular. Even the analysis of sunflowers at 10 minute intervals by the same author shows that the simple sugars vary from about .63% at 11:40 A.M. to about .44% at 11:50 A.M., and back up to about .62% again at 12:00 M. . while the minimum content of simple sugars for the whole period of three hours is .44% and the maximum about .78%.

It seems almost impossible to conceive of carbohydrate concentrations varying as greatly as they do in this work and the work of Clements. From a careful examination of a number of methods for determining reducing and non-reducing sugars it appears that anyone is sufficiently accurate to give reliable results at the time the analyses are made. If, however, the samples had changed any in their carbohydrate content between the time of sampling and the time the analyses were made, it would appear more than probable that they would have occurred between the time the samples were cut and the enzymes killed. This interval of time was much greater in this work than that of Davis and Sawyer[7] or Clements. Clements seems to have been especially careful in getting his samples into 95% alcohol not more than ten minutes after the samples were cut. It is entirely possible that the heating of the material previous to the time of completely killing the enzymes causes great changes in the carbohydrate content.

There may be some other factor thus far not observed that is causing changes in the samples. Webster[31] in studying alcoholic extracts of plants found that amino nitrogen decreased in amount during the storage period ~~from the time the samples are~~ stored. He found no regularity in the decrease, but when potassium nitrate was added to samples of spinach at the time they were preserved, they showed greater decreases in amino nitrogen than spinach without potassium nitrate. Young alfalfa plants which are high in nitrates also had large decreases in aimno nitrogen. He suggested that the amino acids are probably converted to ammonia and a ketone according to the following equation:

$$\begin{array}{l} CH_3 \\ | \\ CH-NH_2 \\ | \\ COOH \end{array} \longrightarrow \begin{array}{l} \quad CH_3 \\ \diagup \\ C=O \\ \diagdown \\ \quad CHO \end{array} + NH_3$$

He further suggested that if the equation represents the true reaction taking place, then it is reasonable to expect an increase in the reducing power of the solutions and an increase in ammonia.

It is not unreasonable to believe that if the work of Webster gives a true indication of changes in amino nitrogen content of extracts when stored, it is possible that other changes are also taking place, and it may give an indication as to why the curves in work of this kind are so very irregular. There seems to be a need for further studies on the methods of preserving plant tissues for various types of analyses and especially those types of analysis dealing with organic materials such as carbohydrates and proteins.

SUMMARY

1. Analyses showing diurnal and seasonal variations in the sugar content of both the sap and tissue of the potato plant are given.

2. Under the conditions of this experiment no differences are seen in the sugar content due to the difference in fertilizer treatment.

3. The curves for both glucose and sucrose seem to run almost horizontal for the early samples, whereas the leaves of the later samples show a minimum concentration of sucrose from 4 A.M. to 8 A.M., and a maximum concentration from noon to 4 P.M. Glucose curves seem to run horizontal in all samples.

4. The percentages of glucose are greater than those of sucrose in all samples of leaves as well as stalks.

5. The concentrations of both sucrose and glucose are higher in the later samples than in the early samples.

6. Greater irregularities are noticed in the analyses of stalks than in the analyses of the leaves.

7. Comparisons between the sugar analyses of tissue and sap of the same plants show the curves to be very much alike and yet not so similar as might be expected.

8. An opinion as to why carbohydrate analyses show such irregularities in variations as noted in this and other work is presented.

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The following tables of data give the detailed analyses from which the curves were drawn. All figures denote percentages of sugars based on the green weight of the plant or the sap, as the case may be.

1st Sampling Leaves 7-23-30

Time of Sampling	No Fertilizer				4-16-0 Fertilizer			
	Glucose Sap	Glucose Tissue	Sucrose Sap	Sucrose Tissue	Glucose Sap	Glucose Tissue	Sucrose Sap	Sucrose Tissue
Mid night	.387	.402	.115	.161	.285	.337	.143	.200
4:00 A.M.	.351	.443	.560	.176		.432		.228
8:00 A.M.	.416	.489	.662	.229	.416	.627	.675	.246
Noon	.493	.810	.381	.261	.480	.701	.421	.280
4:00 P.M.	.450	.515	.321	.313	.649	.620	.246	.158
8:00 P.M.	.398	.541	.199	.365	.617	.600	.119	.264
Mid night	.392	.618	.195	.129	.424	.473	.156	.140

1st Sampling Leaves

Time of Sampling	0-16-8 Fertilizer				4-16-8 Fertilizer			
	Glucose Sap	Glucose tissue	Sucrose Sap	Sucrose tissue	Glucose Sap	Glucose tissue	Sucrose Sap	Sucrose tissue
Mid night	.284	.413	.142	.177	.274	.504	.140	.126
4:00 A.M.	.255	.359	.533	.249	.282	.362	.537	.225
8:00 A.M.	.446	.523	.409	.180	.371	.353	.401	.299
Noon	.532	.536	.398	.317	.415	.671	.442	.203
4:00 P.M.	.442	.645	.326	.174	.369	.512	.272	.222
8:00 P.M.	.518	.507	.176	.281	.482	.449	.183	.251
Mid Night	.209	.462	.152	.111	.203	.529	.296	.090

1st Sampling Stalks 7-23-30

	No Fertilizer				4-16-0 Fertilizer			
Time of Sampling	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.413	.334	0	.156	.371	.313	.106	.119
4:00 A.M.	.313	.191	.189	.318	.397	.256	.110	.167
8:00 A.M.	.646	.478	.143	.202	.561	.345	.066	.217
Noon	.631		.200		.581	.567	.244	.159
4:00 P.M.	.697	.471	.195	.297	.714	.489	.182	.251
8:00 P.M.	.691	.567	.246	.529	.701	.558	.261	.485
Mid night	.897	.694	.104	.160	.613	.507	.044	.132

1st Sampling Stems

	0-16-8 Fertilizer				4-16-8 Fertilizer			
Time of Sampling	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.257	.176	.131	.131	.481	.321	.150	.249
4:00 A.M.	.296	.182	.171	.237	.535	.253	.106	.362
8:00 A.M.	.505	.274	.068	.238	.483	.331	.081	.187
Noon	.483	.398	.201	.222	.438	.368	.186	.166
4:00 P.M.	.487	.336	.131	.235	.489	.390	.143	.272
8:00 P.M.	.642	.393	.118	.297	.548	.398	.108	.238
Mid night	.265	.379	.195	.104	.293	.339	.238	.175

2nd Sampling Leaves 8-12-30

Time of Sampling	No Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	4-16-0 Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.507	.536	.296	.294	.533	.599	.326	.378
4:00 A.M.	.482	.599	.547	.497	.370	.589	.454	.428
8:00 A.M.		.524		.299	.474	.611	.341	.195
Noon	.547	.761	.426	.343	.489	.792	.540	.422
4:00 P.M.	.643	.761	.640	.507	.523	.719	.668	.515
8:00 P.M.	.559	.529	.407	.564	.738	.536	.308	.537
Mid night	.469	.529	.177	.515	.454	.525	.126	.475

Time of Sampling	0-16-8 Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	4-16-8 Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.482	.537	.195	.357	.751	.573	.200	.329
4:00 A.M.	.452	.529	.393	.371	.367	.608	.474	.427
8:00 A.M.	.564	.709	.298	.324	.706	.768	.249	.286
Noon	.530	.857	.300	.329	.569	.701	.653	.522
4:00 P.M.	.785	.497	.471	.538	.561	.558	.729	.772
8:00 P.M.	.746	.509	.258	.438	.675	.682	.409	.315
Mid night	.523	.438	.115	.463	.574	.551	.269	.577

2nd Sampling Stalks 8-12-30

	No Fertilizer				4-16-0 Fertilizer			
Time of Sampling	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.602	.488	.157	.350	.492	.650	.251	.281
4:00 A.M.	.734	.857	.335	.286	.768	.811	.306	.286
8:00 A.M.	.661	.573	.104	.244	.95	.682	.00	.251
Noon	.594	.714	.236	.348	.605	.649	.226	.481
4:00 P.M.	.919	.849	.402	.507	.793	.686	.185	.286
8:00 P.M.	1.054	.694	.165	.447	1.01	.663	.104	.262
Mid night	.935	.857	.00	.393	.899	.559	.00	.325

	0-16-8 Fertilizer				4-16-8 Fertilizer			
Time of Sampling	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.532	.415	.180	.184	.328	.368	.114	.219
4:00 A.M.	.631	.697	.315	.245	.699	.792	.222	.294
8:00 A.M.	.857	.446	.00	.294	.868	.559	.247	.308
Noon	.600	.507	.421	.299	.572	.422	.328	.357
4:00 P.M.	.704	.481	.380	.447	.730	.504	.336	.280
8:00 P.M.	.650	.563	.201	.438	.716	.329	.207	.417
Mid night	.905	.599	.00	.385	.632	.407	.093	.313

3rd Sampling Leaves 9-4-30

	No Fertilizer				4-16-0 Fertilizer			
Time of Sampling	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.496	.589	.291	.538	.439	.441	.396	.400
4:00 A.M.	.585	.810	.247	.212	.455	.523	.314	.263
8:00 A.M.	.344	.700	.400	.203	.457	.631	.509	.0845
Noon0	.447	.714	.699	.666	.537	.677	.735	.630
4:00 P.M.	.549	.797	.874	.730	.726	.894	.771	.621
8:00 P.M.	.447	.769	.523	.671	.420	.834	.560	.450
Mid night	.577	.677	.281	.657	.625	.825	.239	.302

	0-16-8 Fertilizer				4-16-8 Fertilizer			
Time of Sampling	Glucose sap	Blucose tissue	Sucrose sap	Sucrose tissue	Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.336	.507	.380	.354	.428	.497	.443	.476
4:00 A.M.	.673	.654	.295	.328	.490	.797	.369	.311
8:00 A.M.	.389	.604	.442	.393	.376	.609	.498	.368
Noon	.401	.586	.850	.645	.432	.559	.853	.595
4:00 P.M.	.421	.848	.858	.393	.516	1.186	.879	.640
8:00 P.M.	.459	.764	.651	.600	.541	1.108	.683	.581
Mid night	.604	.764	.305	.418	.700	.871	.462	.262

3rd Sampling Stalks 9-4-30

Time of Sampling	No Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	4-16-0 Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	1.146	.746	.350	.690	.770	.640	.307	.476
4:00 A.M.	.779	1.366	.350	.077	.762	.890	.326	.00
8:00 A.M.	.813	.740	.333	.152	.715	.788	.334	.169
Noon	.774	.764	.508	.666	.902	.518	.665	.774
4:00 P.M.	.750	1.282	.635	.450	.684	1.426	.785	.324
8:00 P.M.	.883	.946	.205	.251	.975	1.248	.358	.316
Mid night	.820	.769	.061	.359	1.065	.778	.126	.289

Time of Sampling	0-16-8 Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue	4-16-8 Fertilizer Glucose sap	Glucose tissue	Sucrose sap	Sucrose tissue
Mid night	.555	.619	.290	.289	.611	.403	.325	.522
4:00 A.M.	.862	.757	.326	.402	.835	1.119	.358	.427
8:00 A.M.	.424	.788	.301	.402	.656	.631	.349	.229
Noon	.520	.518	.415	.345	.441	.411	.635	.375
4:00 P.M.	.438	.932	.713	.648	.758	1.501	.656	.363
8:00 P.M.	.731	.937	.324	.321	1.056	1.318	.389	.237
Mid night	1.041	1.032	.243	.246	.827	1.313	.446	.445

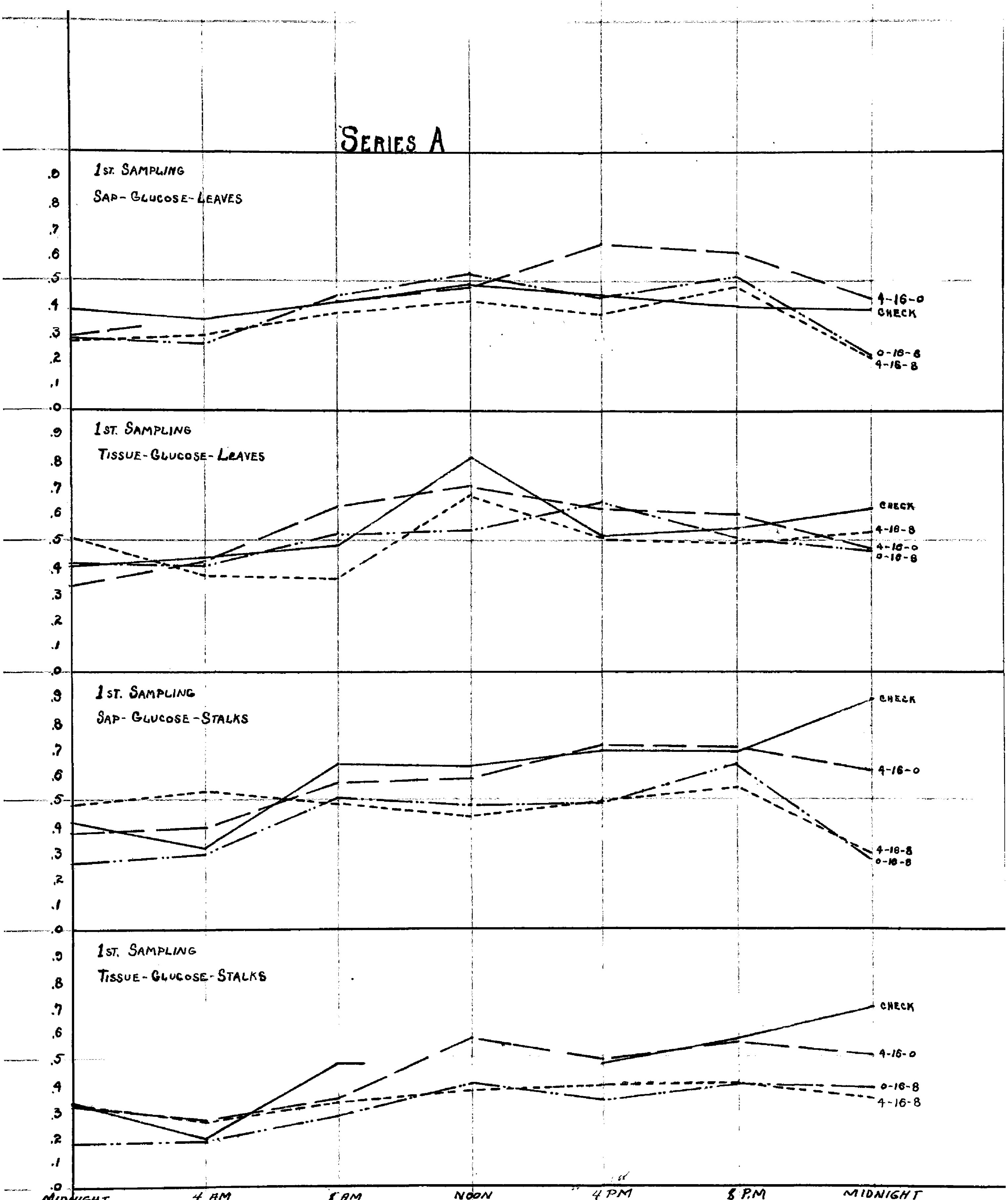

SERIES A
1st. SAMPLING
SAP-GLUCOSE-LEAVES
1st. SAMPLING
TISSUE-GLUCOSE-LEAVES
1st. SAMPLING
SAP-GLUCOSE-STALKS
1st. SAMPLING
TISSUE-GLUCOSE-STALKS
4-16-0
CHECK
0-16-8
4-16-8
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 P.M
MIDNIGHT

FIG. 2

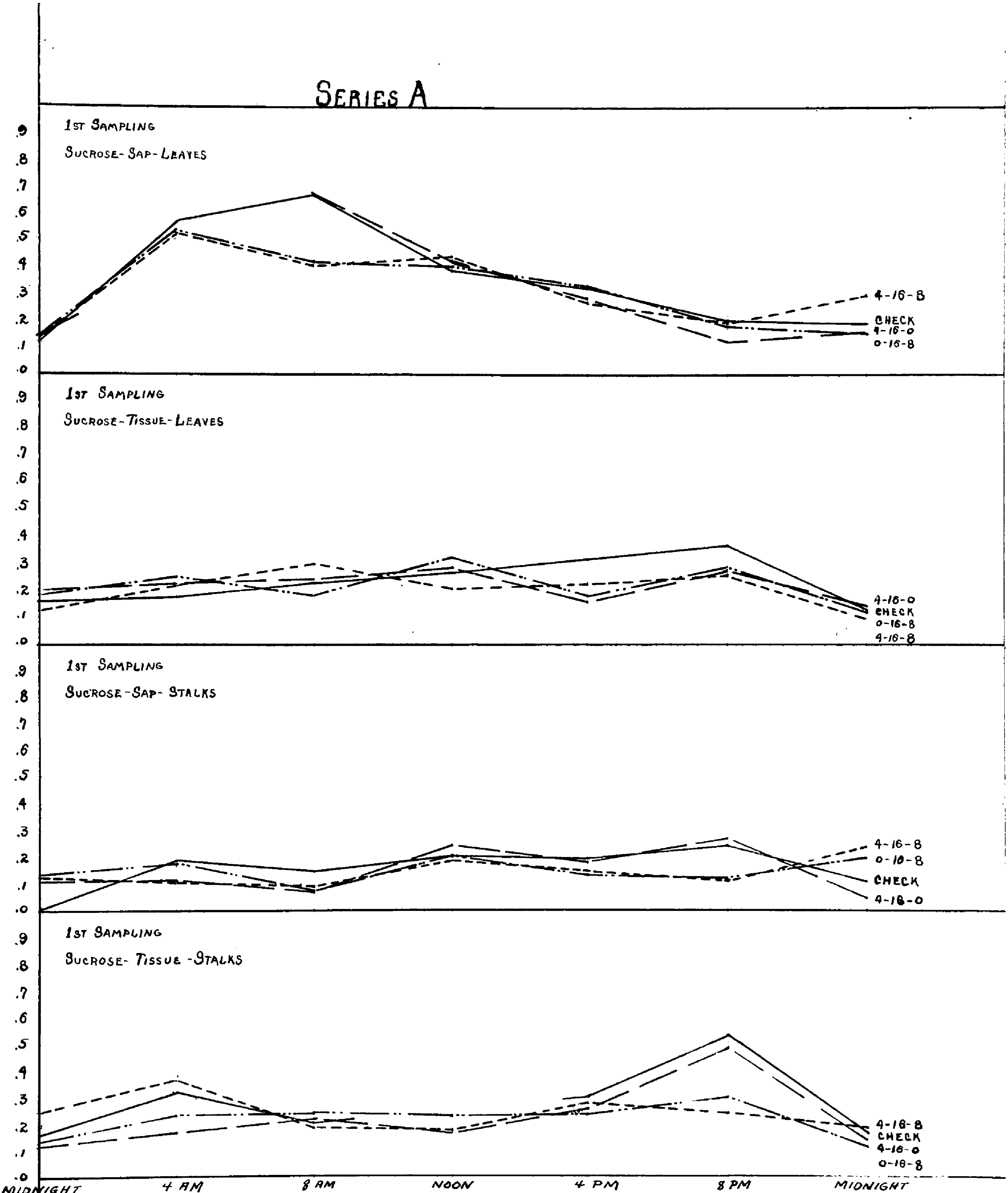

SERIES A
1ST SAMPLING
SUCROSE-SAP-LEAVES
4-16-8
CHECK
4-16-0
0-16-8
1ST SAMPLING
SUCROSE-TISSUE-LEAVES
4-16-0
CHECK
0-16-8
4-16-8
1ST SAMPLING
SUCROSE-SAP-STALKS
4-16-8
0-16-8
CHECK
4-16-0
1ST SAMPLING
SUCROSE-TISSUE-STALKS
4-16-8
CHECK
4-16-0
0-16-8
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 3

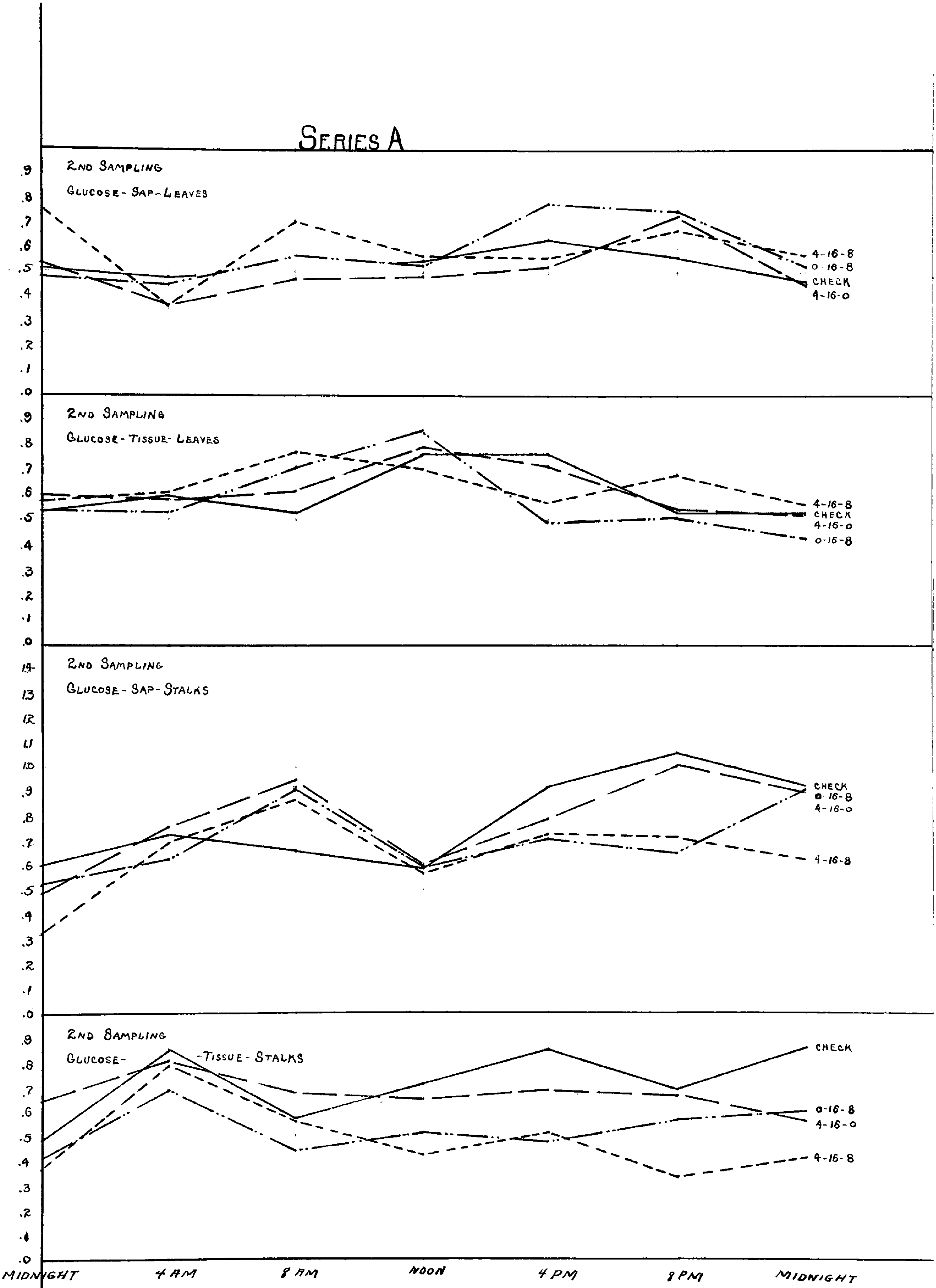

SERIES A
2ND SAMPLING
GLUCOSE - SAP - LEAVES
2ND SAMPLING
GLUCOSE - TISSUE - LEAVES
2ND SAMPLING
GLUCOSE - SAP - STALKS
2ND SAMPLING
GLUCOSE - TISSUE - STALKS
4-16-8
0-16-8
CHECK
4-16-0
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 4

SERIES A

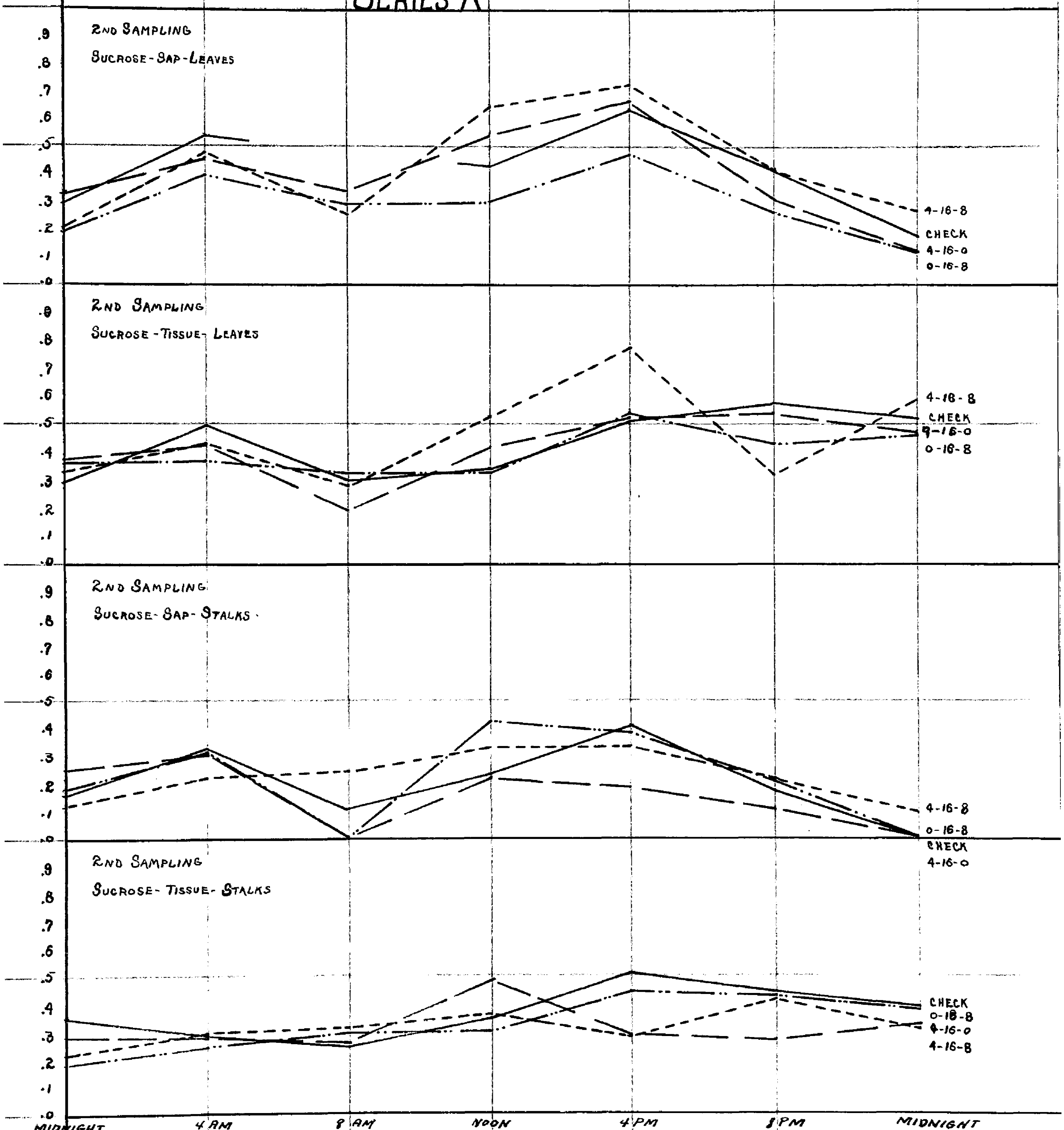
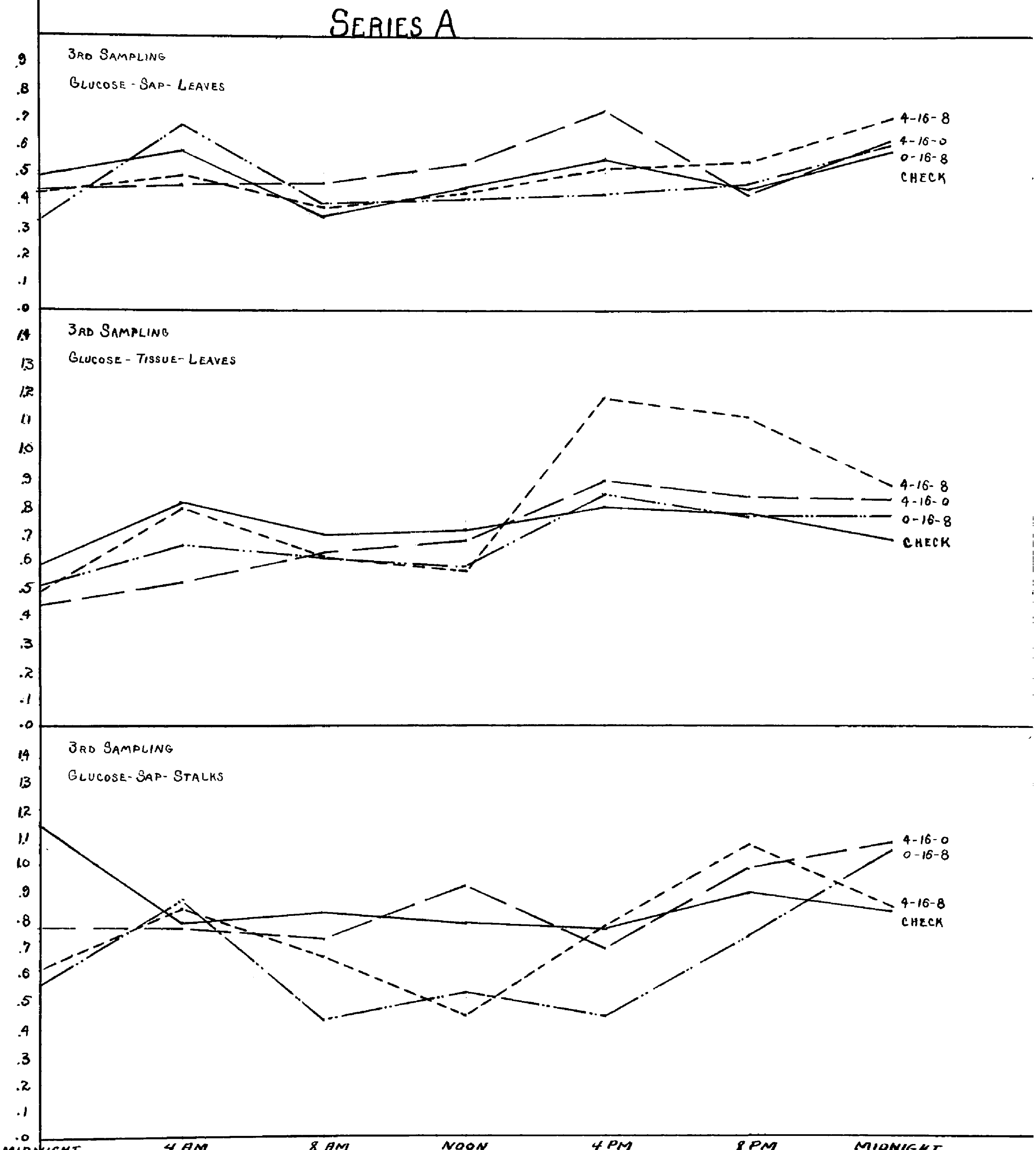


FIG. 5



SERIES A
3RD SAMPLING
GLUCOSE - SAP - LEAVES
4-16-8
4-16-0
0-16-8
CHECK
3RD SAMPLING
GLUCOSE - TISSUE - LEAVES
4-16-8
4-16-0
0-16-8
CHECK
3RD SAMPLING
GLUCOSE - SAP - STALKS
4-16-0
0-16-8
4-16-8
CHECK
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 6

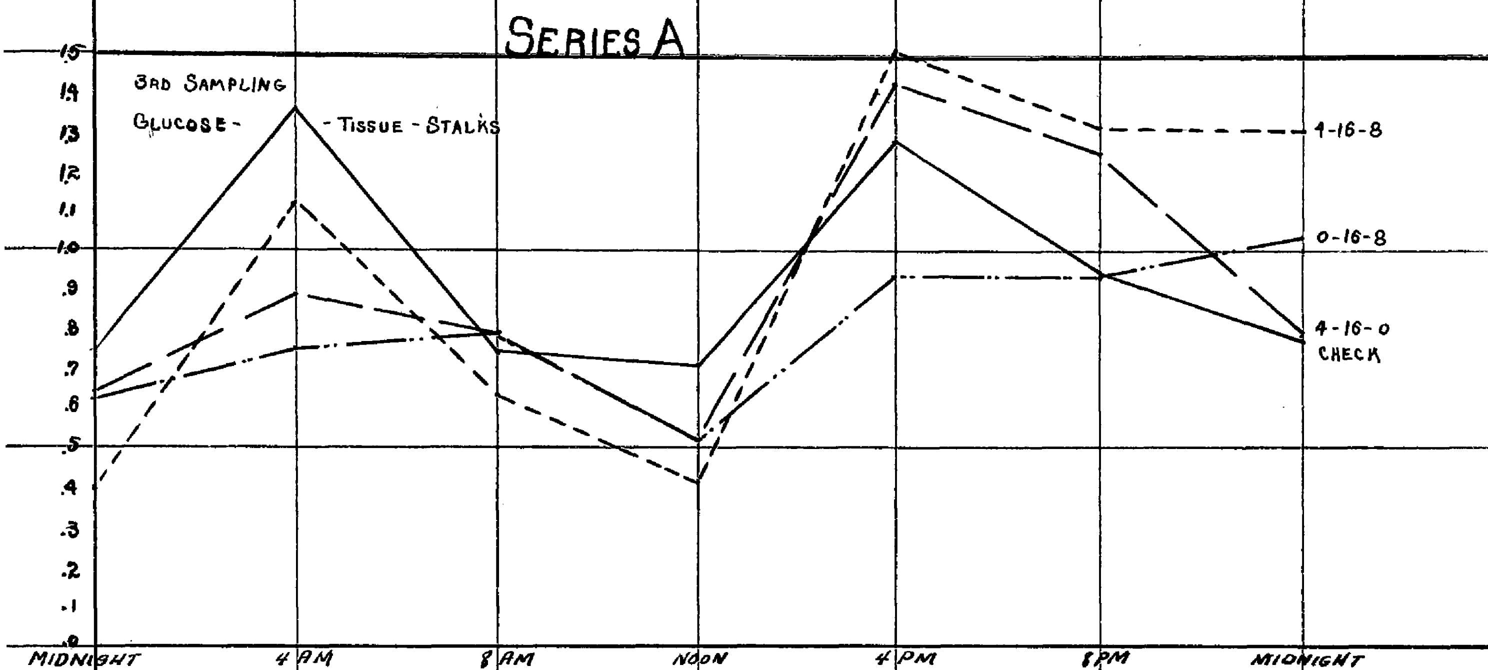
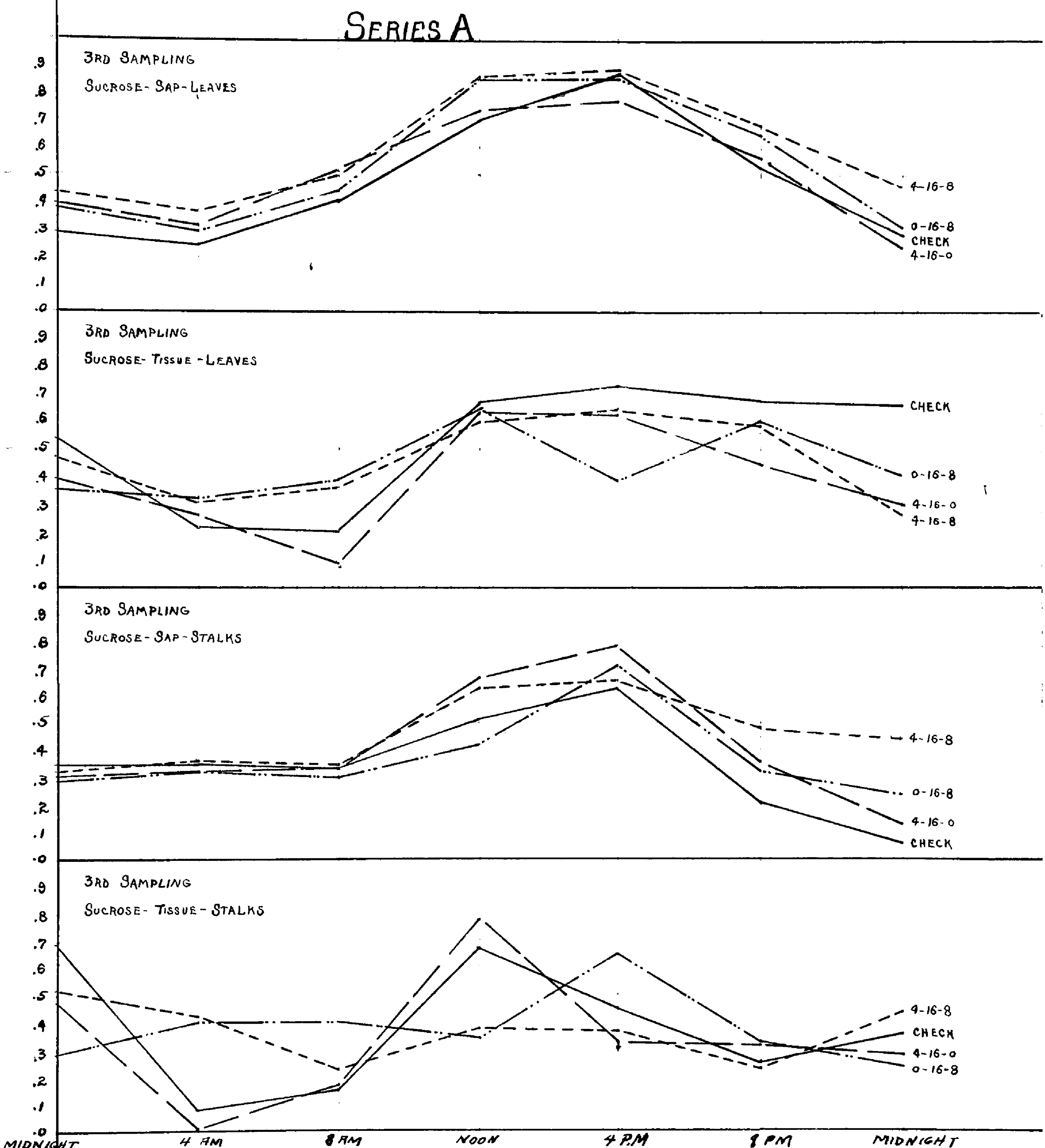
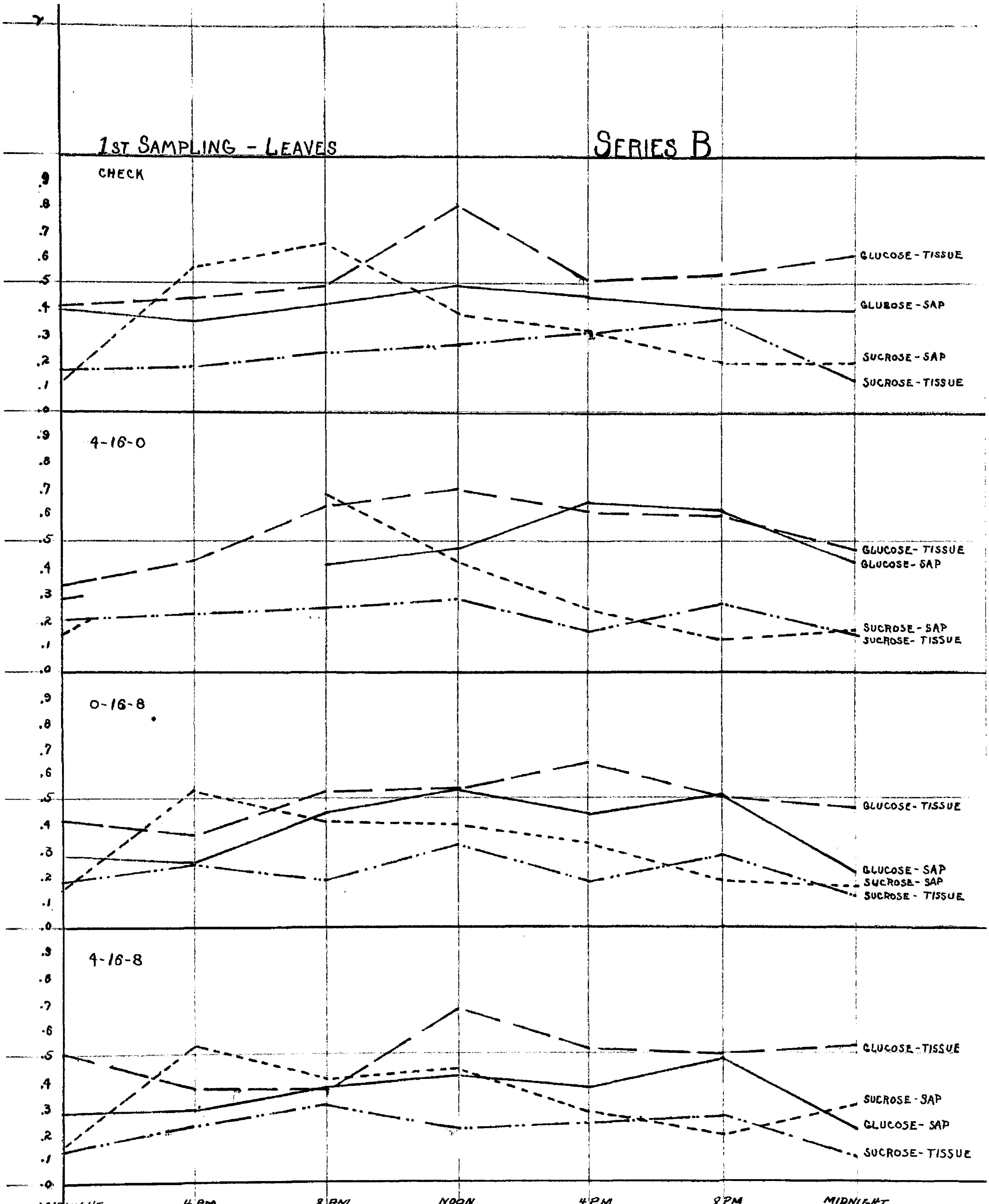

SERIES A
3RD SAMPLING
GLUCOSE - - TISSUE - STALKS
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
4-16-8
0-16-8
4-16-0
CHECK
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 6 (CONT'D)



SERIES A
3RD SAMPLING
SUCROSE- SAP-LEAVES
4-16-8
0-16-8
CHECK
4-16-0
3RD SAMPLING
SUCROSE- TISSUE -LEAVES
CHECK
0-16-8
4-16-0
4-16-8
3RD SAMPLING
SUCROSE- SAP-STALKS
4-16-8
0-16-8
4-16-0
CHECK
3RD SAMPLING
SUCROSE- TISSUE- STALKS
4-16-8
CHECK
4-16-0
0-16-8
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
MIDNIGHT
4 AM
8 AM
NOON
4 P.M
8 PM
MIDNIGHT

FIG. 7



1st SAMPLING - LEAVES
SERIES B
CHECK
4-16-0
0-16-8
4-16-8
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
GLUCOSE - TISSUE
GLUCOSE - SAP
SUCROSE - SAP
SUCROSE - TISSUE
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 8

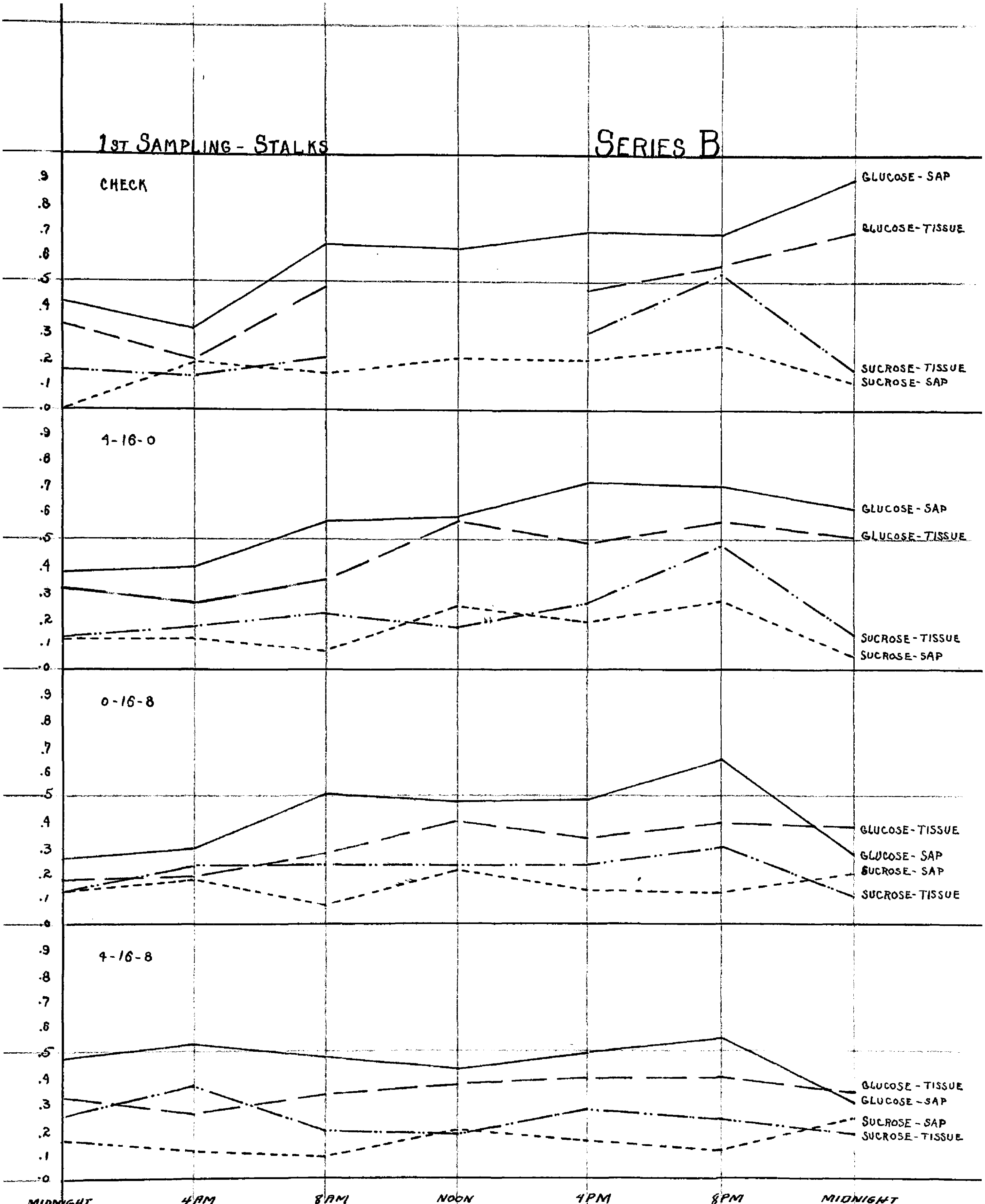

1ST SAMPLING - STALKS
SERIES B
CHECK
4-16-0
0-16-8
4-16-8
GLUCOSE - SAP
GLUCOSE - TISSUE
SUCROSE - TISSUE
SUCROSE - SAP
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 9

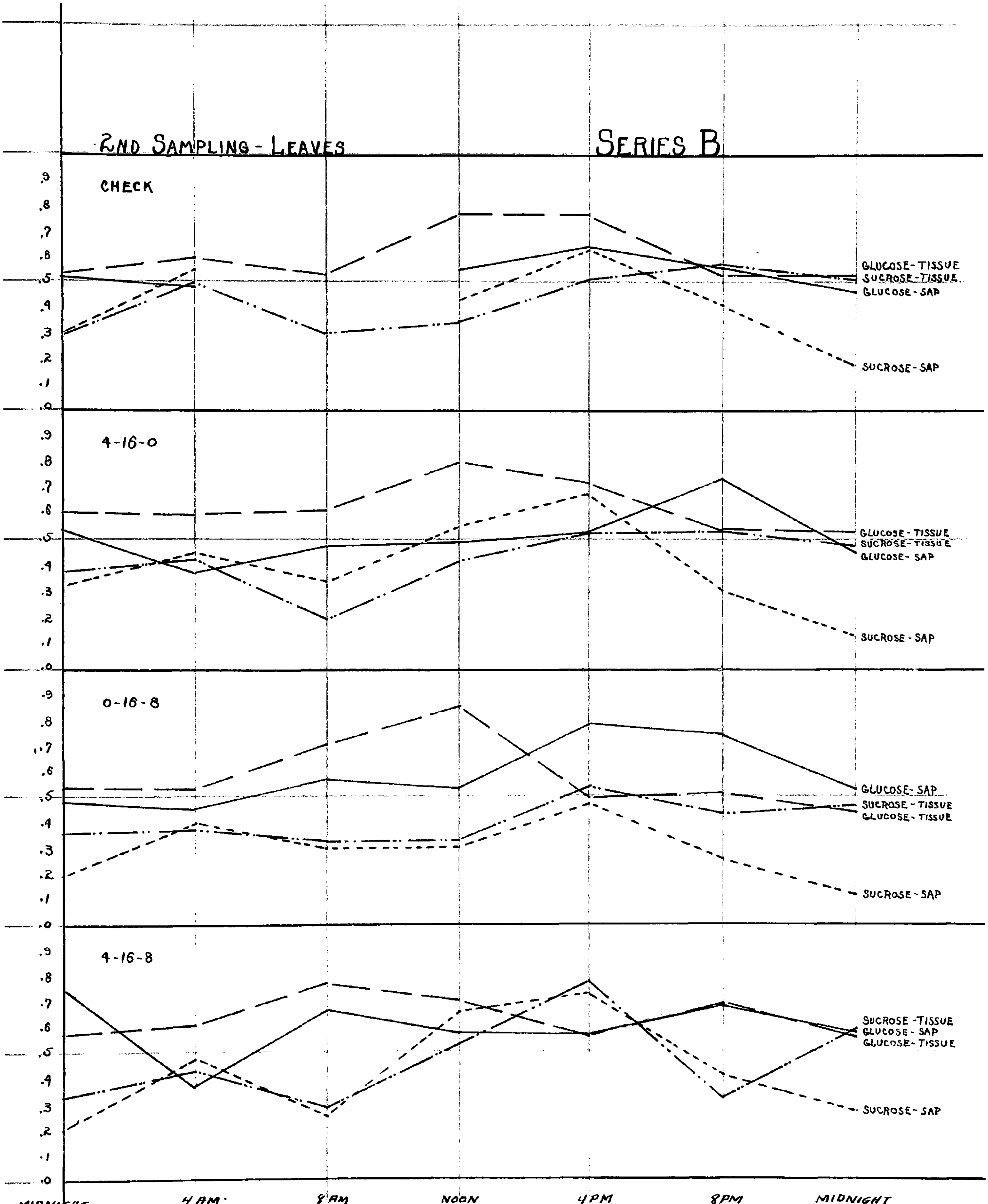
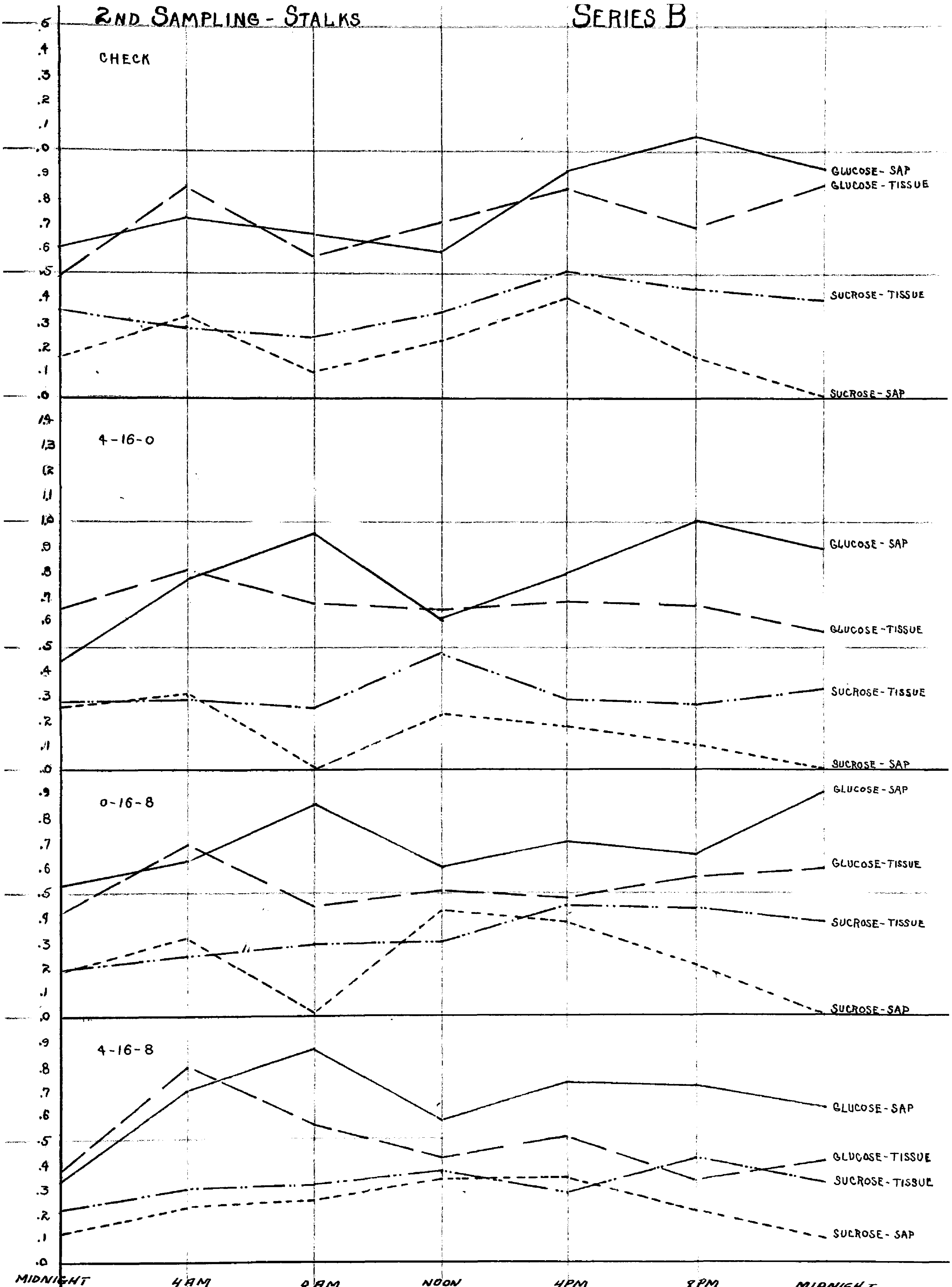

2ND SAMPLING - LEAVES
SERIES B
CHECK
4-16-0
0-16-8
4-16-8
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
GLUCOSE-TISSUE
SUCROSE-TISSUE
GLUCOSE-SAP
SUCROSE-SAP
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 10



2ND SAMPLING - STALKS
SERIES B
CHECK
4-16-0
0-16-8
4-16-8
GLUCOSE - SAP
GLUCOSE - TISSUE
SUCROSE - TISSUE
SUCROSE - SAP
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 11

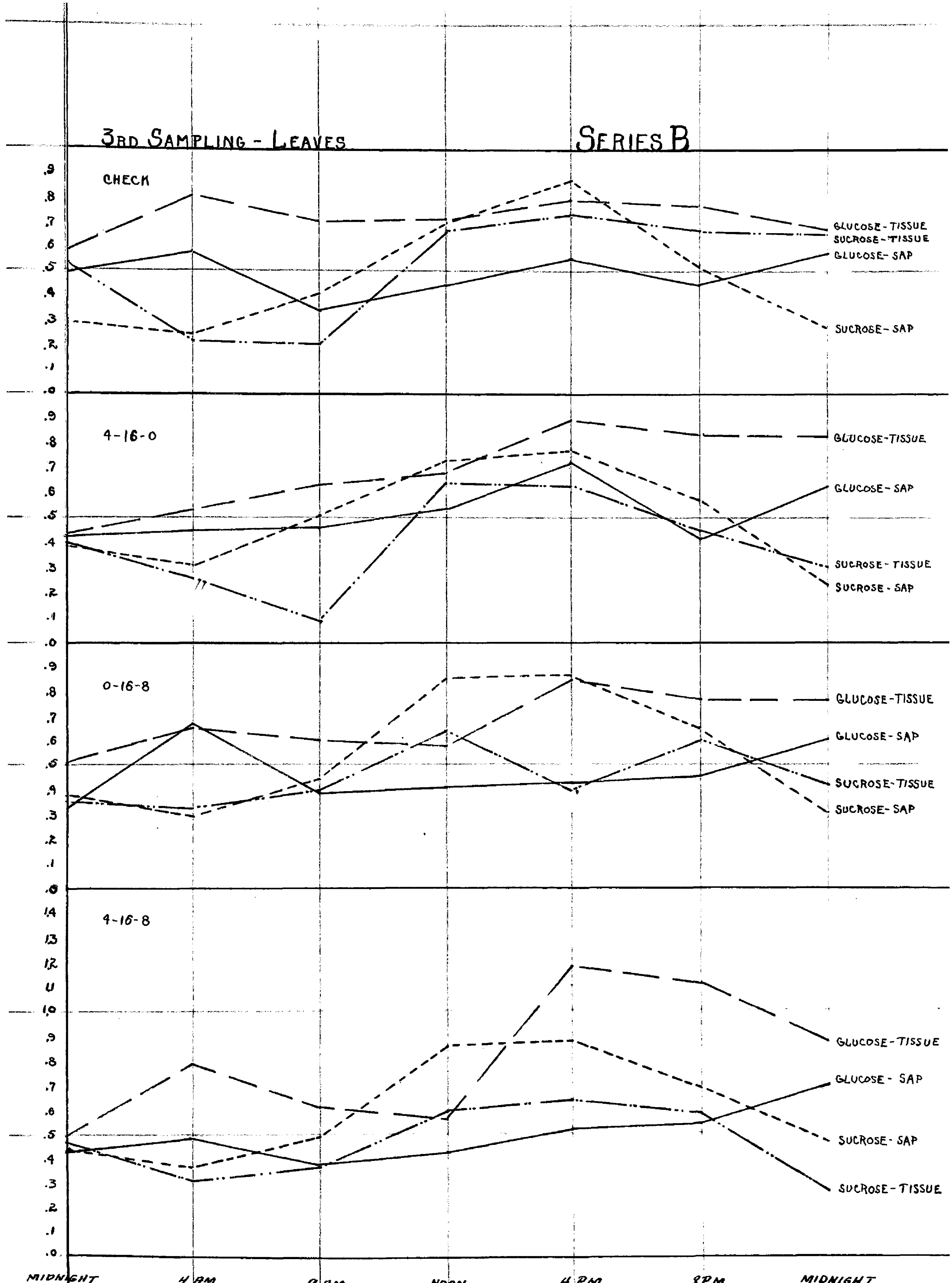

3RD SAMPLING - LEAVES
SERIES B
CHECK
4-16-0
0-16-8
4-16-8
GLUCOSE-TISSUE
SUCROSE-TISSUE
GLUCOSE-SAP
SUCROSE-SAP
.0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
1.1
1.2
1.3
1.4
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 P.M
MIDNIGHT

FIG. 12

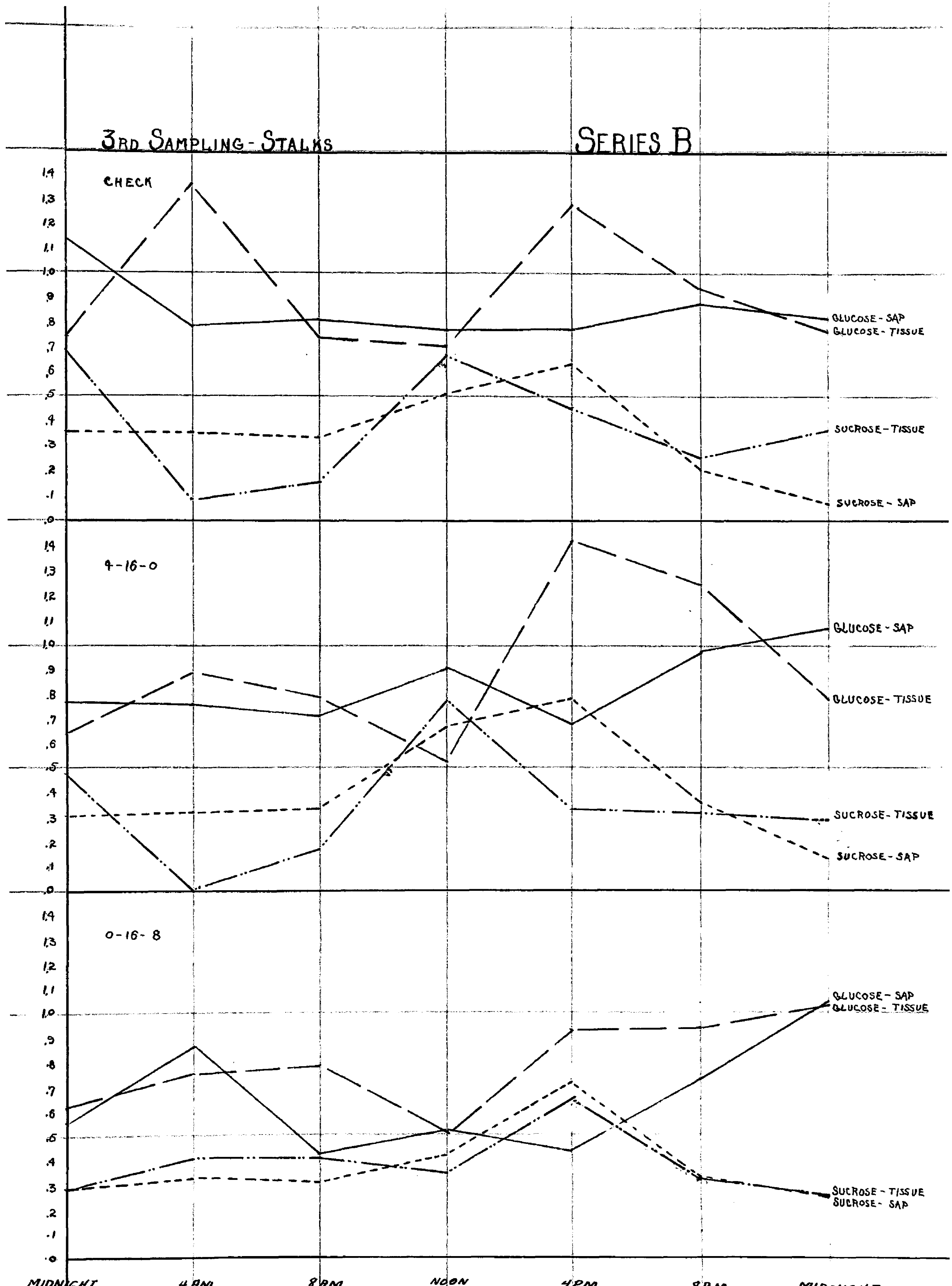
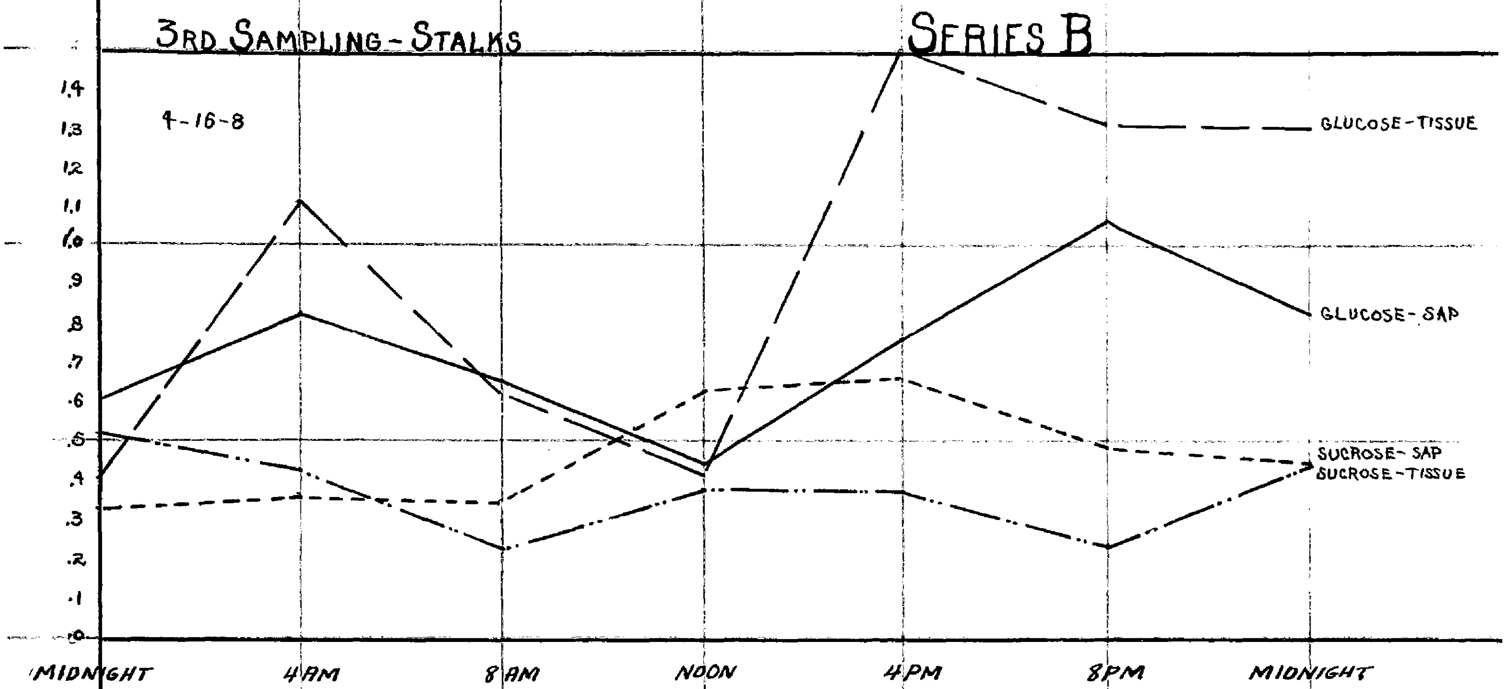

3RD SAMPLING - STALKS
SERIES B
CHECK
4-16-0
0-16-8
GLUCOSE - SAP
GLUCOSE - TISSUE
SUCROSE - TISSUE
SUCROSE - SAP
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 P.M
MIDNIGHT

FIG. 13



3RD SAMPLING - STALKS
SERIES B
4-16-8
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
GLUCOSE-TISSUE
GLUCOSE-SAP
SUCROSE-SAP
SUCROSE-TISSUE
MIDNIGHT
4AM
8 AM
NOON
4PM
8PM
MIDNIGHT

FIG. 13 (CONT'D)

SERIES C

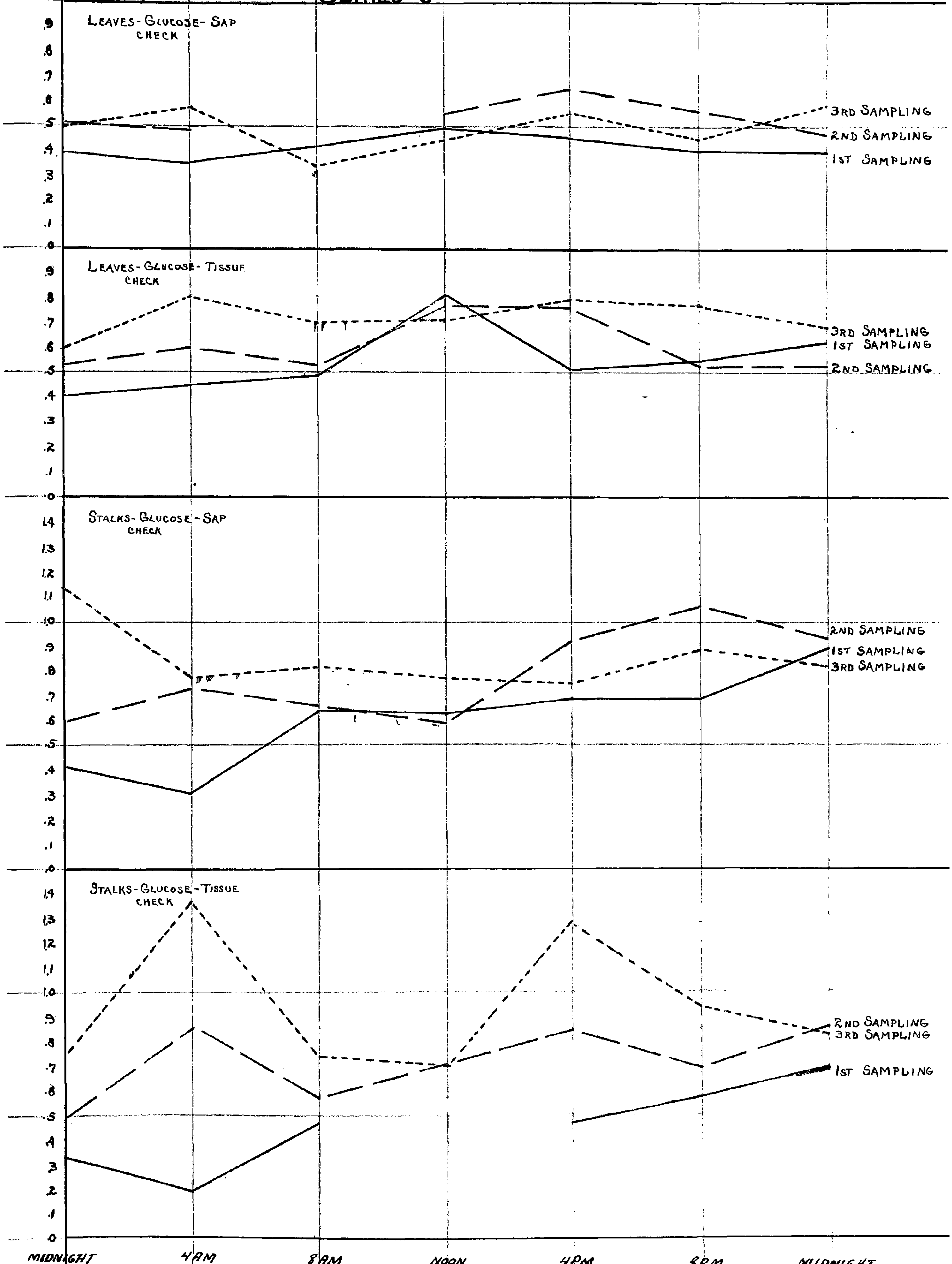


FIG. 14

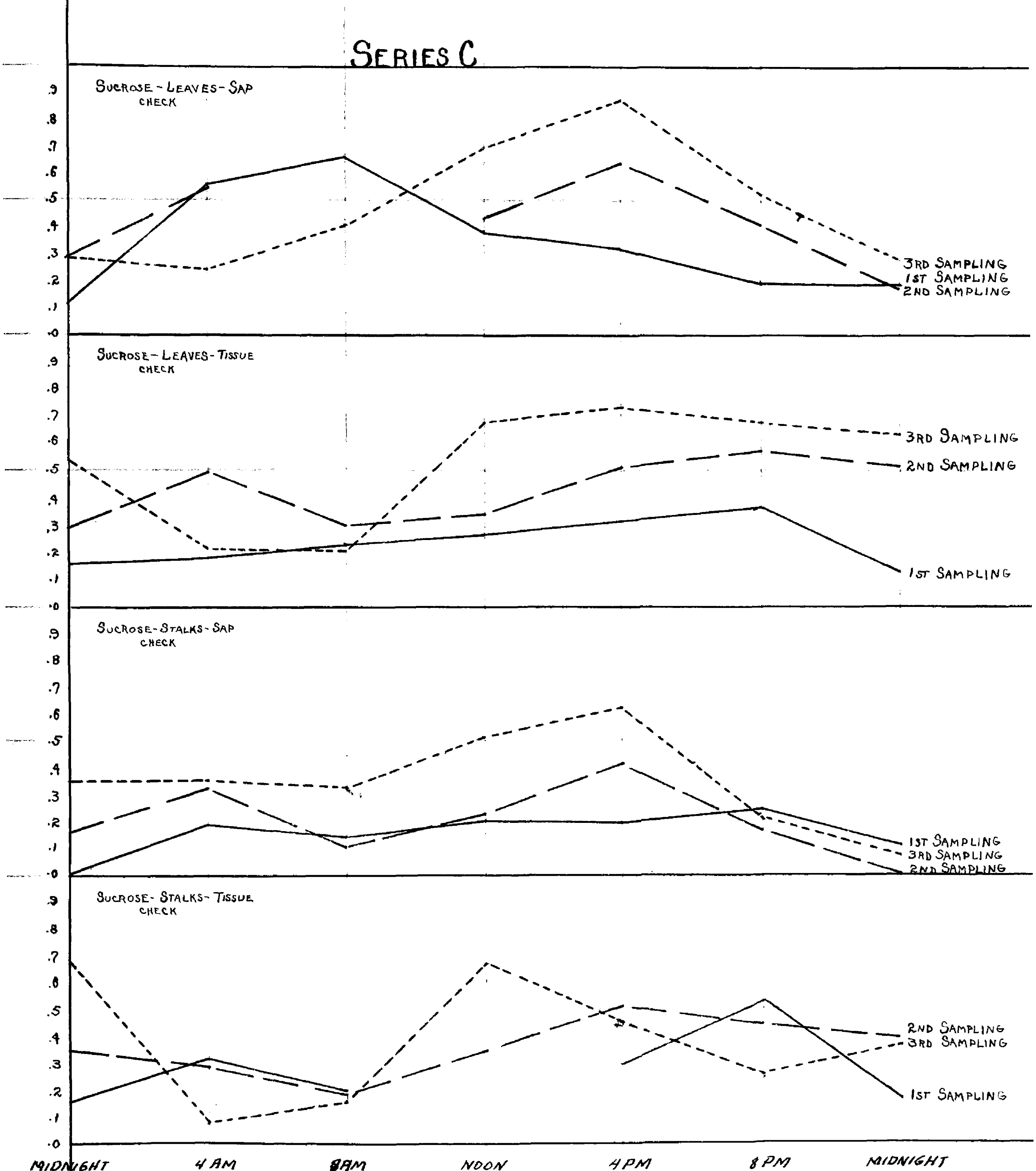
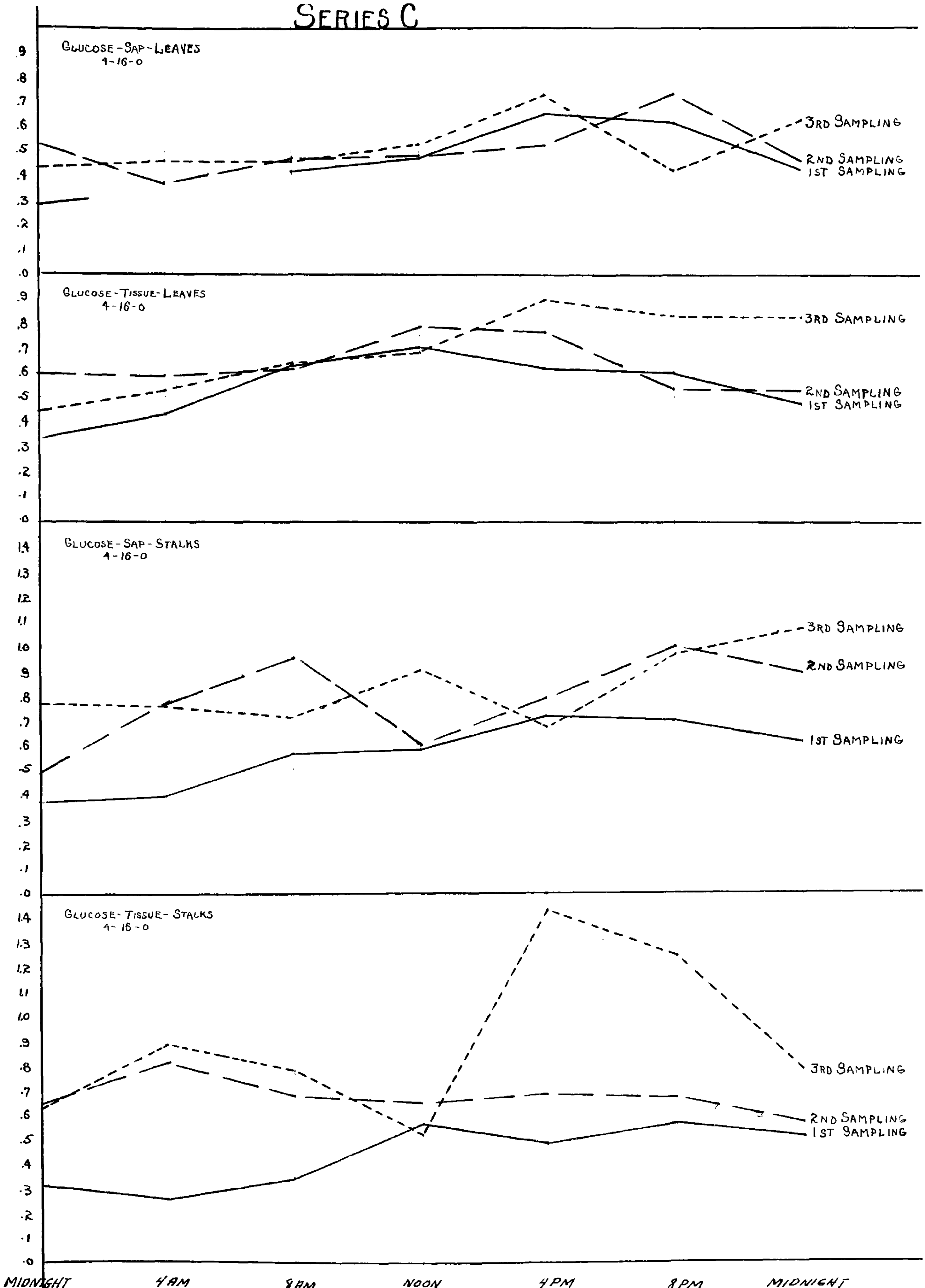

SERIES C
SUCROSE - LEAVES - SAP
CHECK
3RD SAMPLING
1ST SAMPLING
2ND SAMPLING
SUCROSE - LEAVES - TISSUE
CHECK
3RD SAMPLING
2ND SAMPLING
1ST SAMPLING
SUCROSE - STALKS - SAP
CHECK
1ST SAMPLING
3RD SAMPLING
2ND SAMPLING
SUCROSE - STALKS - TISSUE
CHECK
2ND SAMPLING
3RD SAMPLING
1ST SAMPLING
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
MIDNIGHT
4 AM
8AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 15



SERIES C
GLUCOSE - SAP - LEAVES
4-16-0
GLUCOSE - TISSUE - LEAVES
4-16-0
GLUCOSE - SAP - STALKS
4-16-0
GLUCOSE - TISSUE - STALKS
4-16-0
3RD SAMPLING
2ND SAMPLING
1ST SAMPLING
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 16

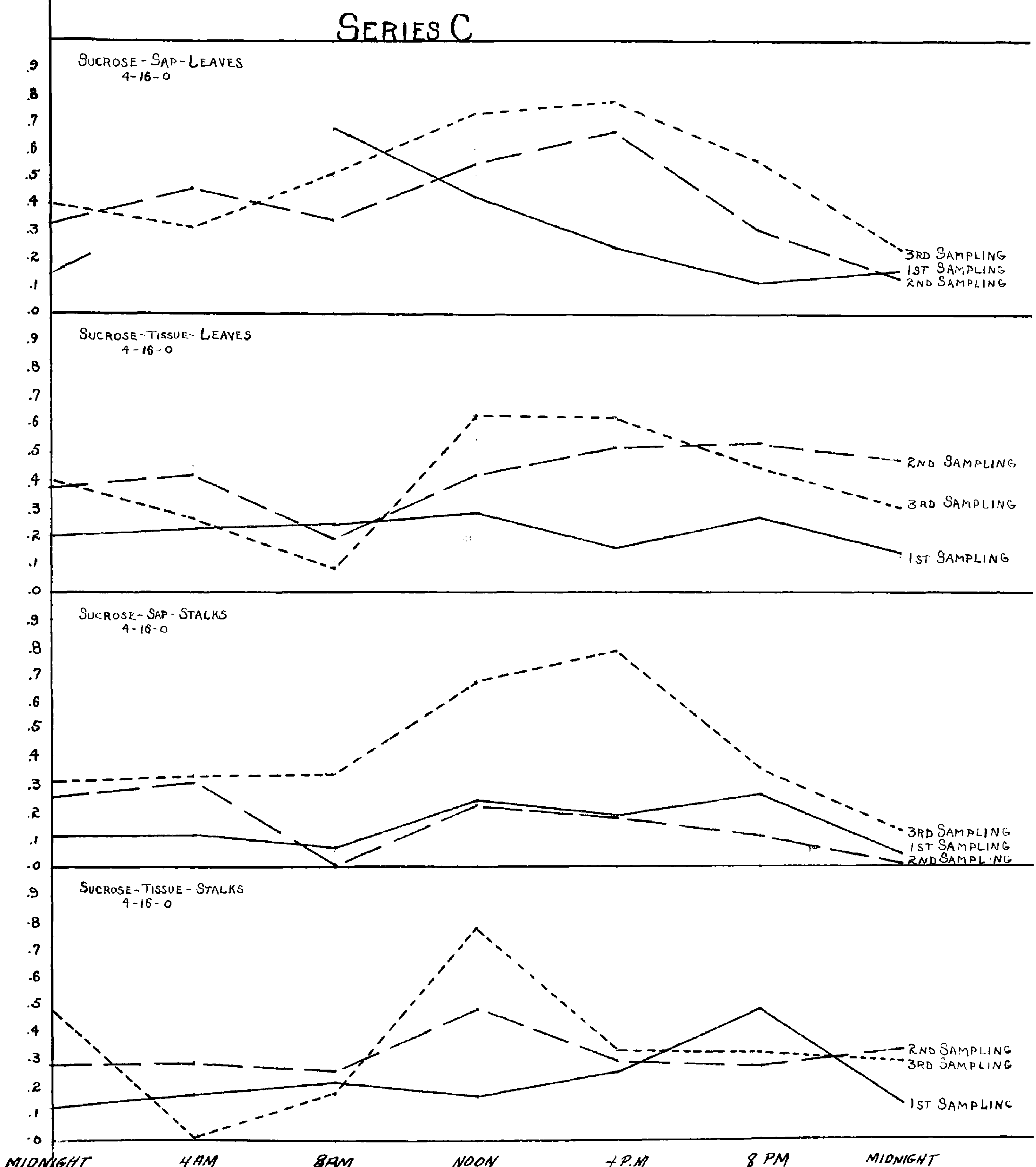

SERIES C
SUCROSE - SAP - LEAVES
4-16-0
SUCROSE - TISSUE - LEAVES
4-16-0
SUCROSE - SAP - STALKS
4-16-0
SUCROSE - TISSUE - STALKS
4-16-0
3RD SAMPLING
1ST SAMPLING
2ND SAMPLING
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
MIDNIGHT
4 AM
8 AM
NOON
4 P.M
8 PM
MIDNIGHT

FIG. 17

SERIES C

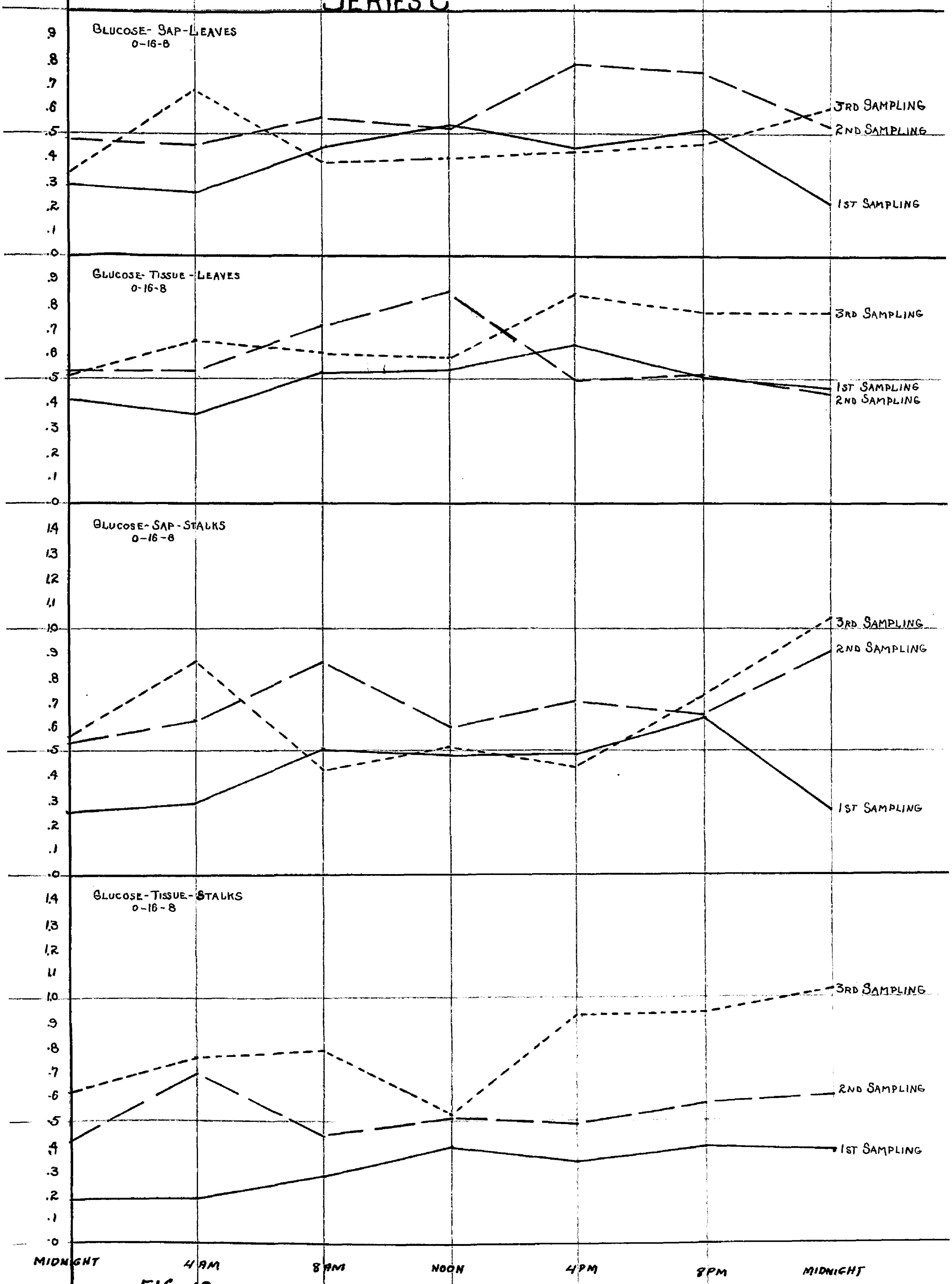
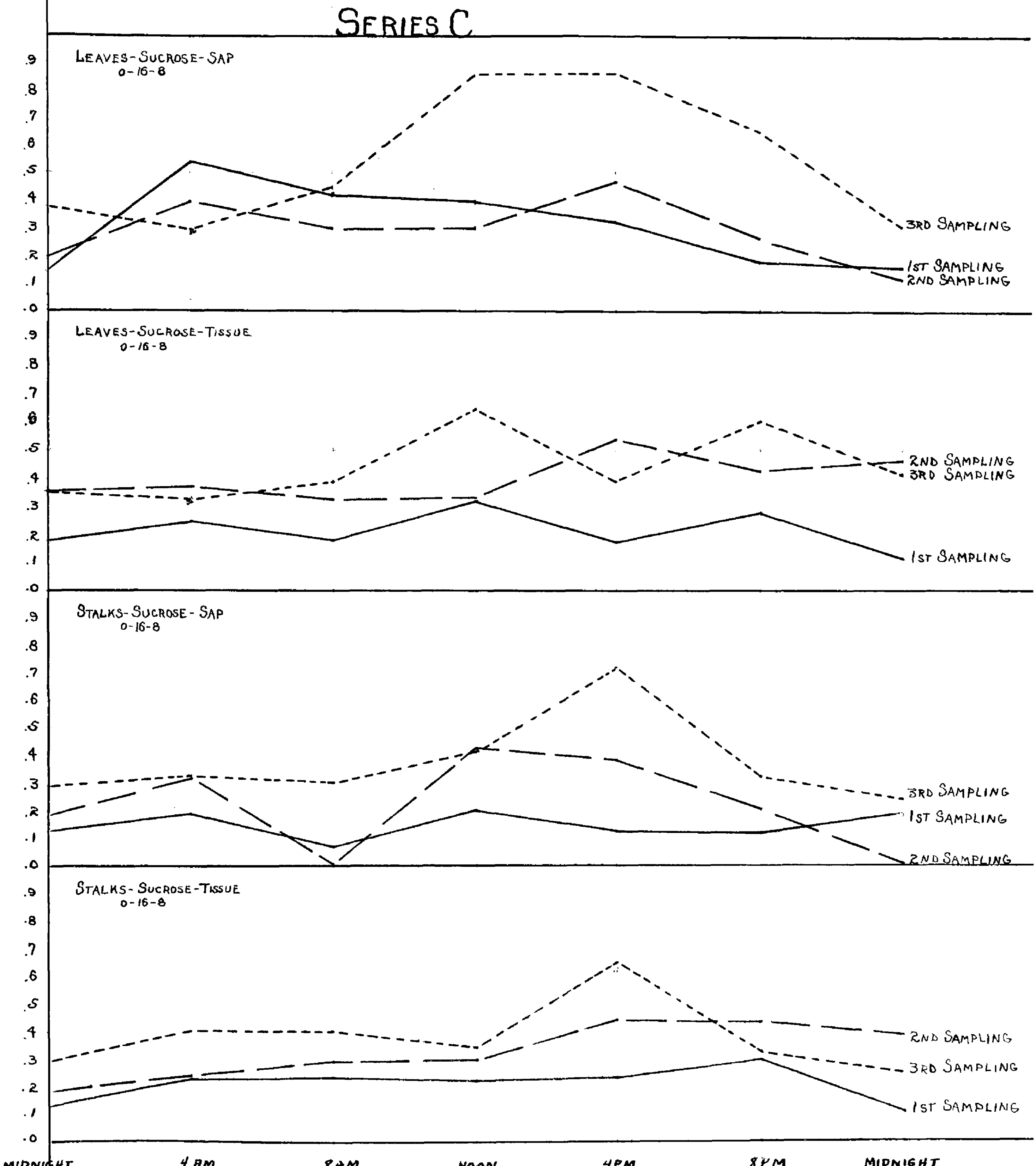


FIG. 18



SERIES C
LEAVES-SUCROSE-SAP
0-16-8
LEAVES-SUCROSE-TISSUE
0-16-8
STALKS-SUCROSE-SAP
0-16-8
STALKS-SUCROSE-TISSUE
0-16-8
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
3RD SAMPLING
1ST SAMPLING
2ND SAMPLING
MIDNIGHT
4 AM
8 AM
NOON
4 PM
8 PM
MIDNIGHT

FIG. 19

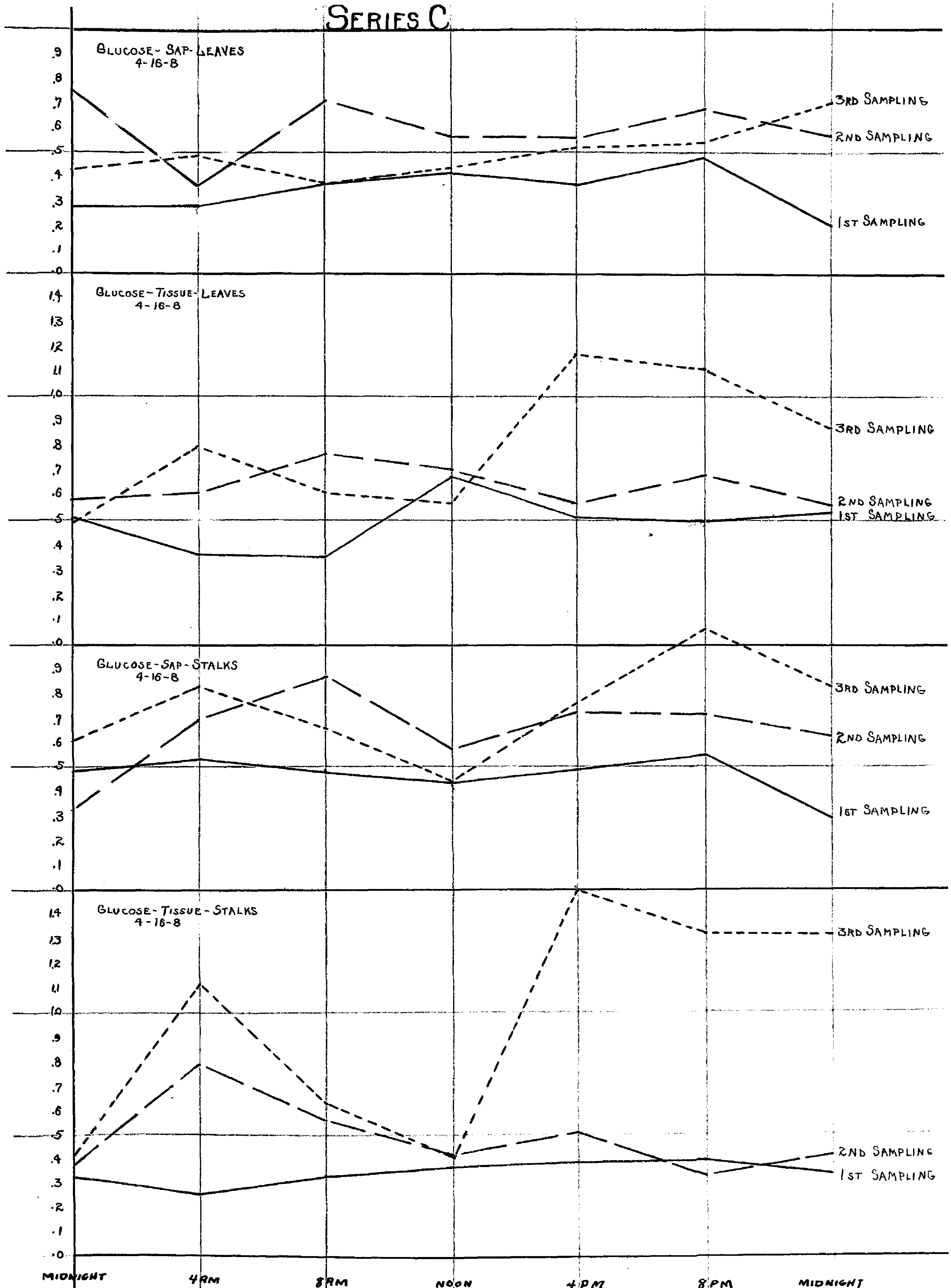
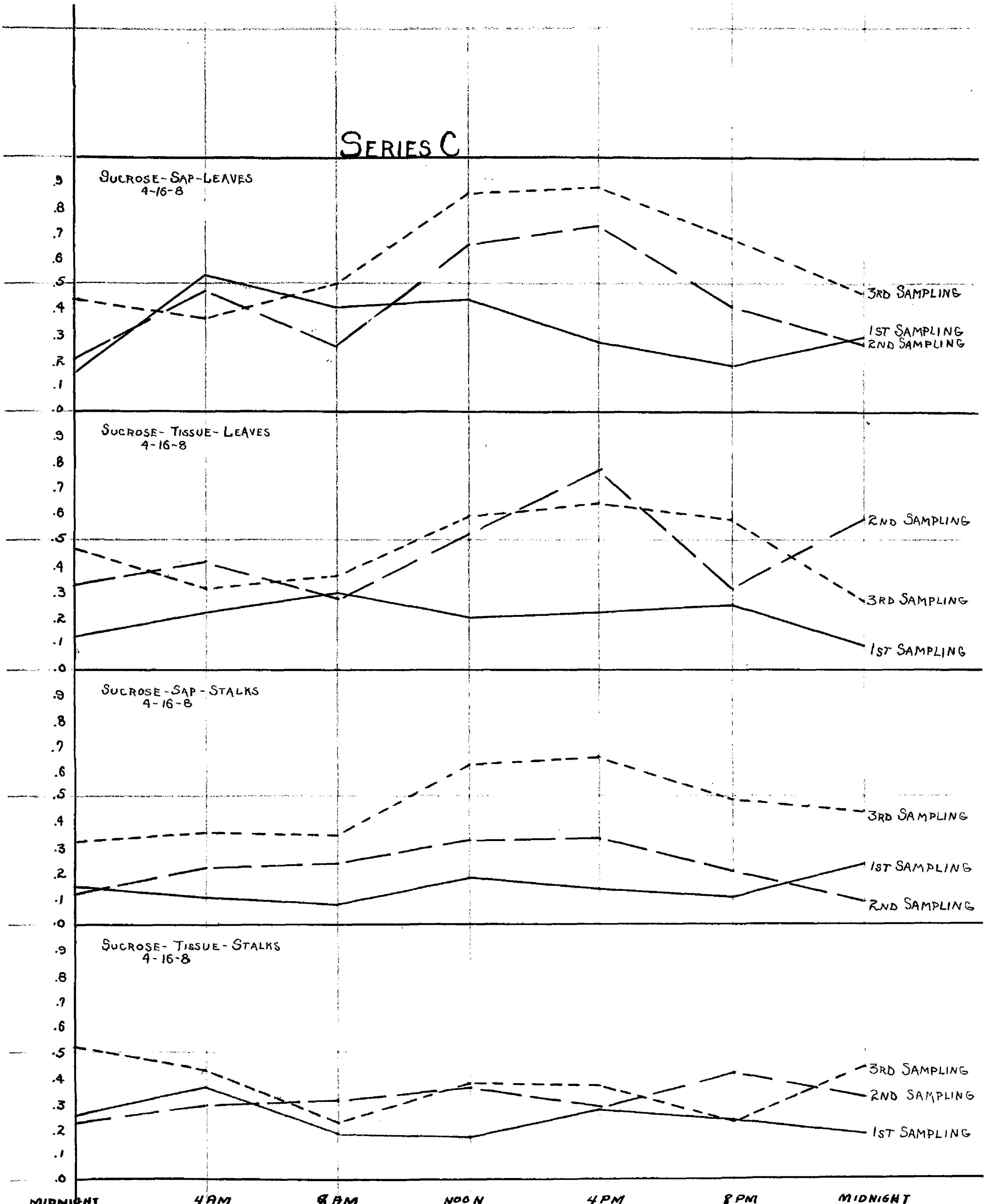

SERIES C
GLUCOSE-SAP-LEAVES
4-16-8
GLUCOSE-TISSUE-LEAVES
4-16-8
GLUCOSE-SAP-STALKS
4-16-8
GLUCOSE-TISSUE-STALKS
4-16-8
3RD SAMPLING
2ND SAMPLING
1ST SAMPLING
MIDNIGHT
4AM
8AM
NOON
4PM
8PM
MIDNIGHT

FIG. 20



SERIES C
SUCROSE-SAP-LEAVES
4-16-8
SUCROSE-TISSUE-LEAVES
4-16-8
SUCROSE-SAP-STALKS
4-16-8
SUCROSE-TISSUE-STALKS
4-16-8
.9
.8
.7
.6
.5
.4
.3
.2
.1
.0
3RD SAMPLING
1ST SAMPLING
2ND SAMPLING
MIDNIGHT
4AM
8AM
NOON
4PM
8PM
MIDNIGHT

FIG. 21