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Entitled THE EFFECT OF ENVIRONMENTAL FACTORS ON THE CARBOHYDRATES AND NUTRIENT LEVELS OF CREEPING BENTGRASS (Agrostis palustris)

Complies with the University regulations and that it meets the accepted standards of the Graduate School with respect to originality and quality

For the degree of:

Master of Science

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THE EFFECT OF ENVIRONMENTAL FACTORS ON THE CARBOHYDRATE AND NUTRIENT LEVELS OF CREEPING BENTGRASS (*Agrostis palustris*)

A Thesis

Submitted to the Faculty

of

Purdue University

by

Edward E. Jordan

In Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

June 1959

ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Dr. W. H. Daniel for his patience and helpful counseling. For encouragement and timely advice, appreciation is expressed also to Dr. B. O. Blair and Professor S. R. Miles.

Special acknowledgement is given to Dr. M. R. Teel for his stimulating ideas and to a fellow student, J. B. Beard, whose cooperation during the latter part of the work was invaluable.

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ABSTRACT

Jordan, Edward E., M. S., Purdue University, June 1959. The Effect of Environmental Factors on the Carbohydrate and Nutrient Levels of Creeping Bentgrass, (Agrostis palustris).

PART I GREENHOUSE

Common creeping bentgrass, Agrostis palustris, is widely used for putting green surfaces in temperate northern United States. Its performance is highly dependent on proper management, especially during the warmer portions of the summer.

Temperature is considered to be a major environmental factor during such critical times. It seems to alter the enzymatic systems within the plant tissue. These enzymatic systems carry on the major functions of the plant, some of which are critically affected by temperature levels such as respiration, photosynthesis, and growth or tissue assimilation.

Fructose, a simple hexose sugar, is an excellent indicator for respiration and photosynthesis in cool season grasses. The level of fructose present is the net or balance between these competing systems. Growth when measured as dry weight is an excellent indicator of of the assimilation function.

Established bentgrass sod was grown under constant light, moisture, and nutrients at temperatures of 60, 70, and 80 degrees F. The fructose levels in leaf tissues and the dry weights of the leaf clippings indicated that 70°F was the best of the three temperatures for leaf tissue development. The 60° and 80° temperatures imposed blocks upon the systems which were involved in the assimilation process. This decreased the dry weights of clippings; but, at the same time, there was a measurable build up of fructose levels in the leaves, since respiration and photosynthesis systems were functioning at near normal rates.

ABSTRACT
(continued)

PART II FIELD STUDY

Common creeping bentgrass was grown in the field under natural putting green conditions. Thirty-eight different ecological and physiological factors to which the grass was subjected were simultaneously measured. The relationships of each upon the other were observed and two were selected for further analysis.

One study compared yields of clippings against seven ecological and eight physiological parameters. Using all fifteen variables, 73.1% of the variations in yield could be accounted for. However, by using the five most important variables: reducing sugar levels, per cent light brown roots at five inches, fructose levels, maximum soil temperature at one-half inch, and moisture at one inch, 52.5% of the variations in yield could be accounted for.

A second study was run using this same data, but compared leaf fructose levels against the same fifteen variables. Using all fifteen parameters, 88.6% of the variations could be accounted for. However, by using the five most important variables: maximum soil temperature at one-half inch, soil moisture at one inch, yield,

light intensity, and per cent white roots at five inches, 70.7% of the variations in fructose levels could be accounted for.

THE EFFECT OF ENVIRONMENTAL FACTORS ON THE CARBOHYDRATE AND NUTRIENT LEVELS OF CREEPING BENTGRASS (*Agrostis palustris*)

INTRODUCTION

Common creeping bentgrass, *Agrostis palustris*, is a perennial stoloniferous grass, used extensively for putting greens and fine lawns in temperate northern United States. It requires intensive management to maintain its fine grass qualities, especially during the warm and humid periods of the year. Greenkeepers have long realized that high temperatures can destroy or weaken established sods and thus allow and ingression of less desirable grasses and weeds. But, little is known about the actual effects of temperature on the physiological functions within the bentgrass tissue.

Previous studies using *vitro* reactions with a number of different plant tissues have shown some general relationships. But, these studies may be misleading, since they destroy the physical order and structure of cells. These disruptions may critically affect rates of reactions. In general, increases in temperature accelerate respiration, but fail to increase rates of photosysthesis. Therefore, at some temperature the rate of respiration will, theoretically, begin to exceed photo-

synthesis. It will create a condition in which the plant must mobilize its storage compounds to continue to respire and maintain life.

In cool season grasses, fructose, a simple hexose sugar, is the major sugar and constituent of the storage compound, fructan. It was theorized that in vivo studies, fructose could be used as an indicator to measure the balance between the photosynthesizing, respiratory, and storage systems, and the effectiveness of the assimulation system could be measured by dry weight increases in new tissue.

Two methods were used to study the effect of temperature upon the physiological systems.

1. Plants were grown at selected constant temperatures, and the fructose levels and growth rates were recorded. The effect of temperature was considered to be one of direct nature with no consideration given to indirect effects.
2. Temperature along with other ecological and physiological factors was measured as it existed under natural conditions. The relative importance of each was given consideration when their combined effect was compared against yield. Their effect was then compared with fructose levels.

REVIEW OF THE LITERATURE

Two general methods are used in studying the effect of an environmental factor upon one or a group of physiological systems. The most commonly used is a simple, direct comparison between changes of the factor and the changes in the physiological system. Other factors are maintained or considered constant, and all changes of the physiological system are considered to be a direct result of the changes of the varied factor. Little consideration is given to interaction among the related physiological systems.

A second approach is to measure as many natural or artificial changes of the factors and as many corresponding changes of the physiological systems as is feasible. The advantage is that interactions may be studied. The disadvantages is the number of samples which must be taken to give statistical significance to the conclusions.

These methods and biostatistical techniques are demonstrated by Houseman and others (15, 16, 5, 32). They consider every factor as well as every physiological function to be inter-related in either a positive or negative manner. The magnitude of the relationship can be approached by statistical methods.

Norman (25) strongly endorses the biochemical approach to determining the relationships among ecological

factors, differential organ growth, and internal physiological systems. Each constituent's relation is considered in a number of plant tissues. However, in order to determine whether this relationship exists in bentgrass, it is necessary to determine which one of the carbohydrates serves as the energy and structural substrate. Archbold(1) and Weinmann (41) stress the importance of the fructose polymers. The name, fructan, is frequently used to describe the specific fructose polymer found in monocotyledons; fructosan is used to describe all of the fructose polymers. Whistler (44) elaborates on these classifications and adopts the term, fructan, for only those polymers found in monocotyledons. The chemical descriptions of the polymers are given by McDonald (21).

DeCugnac (6), in analyzing thirty-eight species of grass for their various photosynthate and storage compounds, was able to separate the grasses into two catagories. Graminees levulferes are the grasses which store fructan but contain no starch; however, there may be sucrose present in their tissues. Graminees sacchariferes are the grasses which store sucrose and may have starch present but have no fructan polymers.

A general ecological separation may be made upon these two groups. The ones which are adapted to the cooler climates, called cool-season grasses, are found in the

Graminees - levulferes - group.

Temperature will effect the balance between the concentration of the simple photosynthate and the complex storage carbohydrate. Winkler (45), Hopkins (14), and others have shown that low temperatures generally favor the transformations from complex to simple sugar, and that high temperatures favor the transformations from simple to complex sugars.

The greatest effect of temperature seems to be upon the balance between the two major physiological systems of respiration and photosynthesis. Platenius (28) has shown that respiration rates within the range of 10° to 30°C are usually doubled for every ten-degree increase in temperature. While Thomas and Hill (37) and Childers and White (4) have found that within this 10°-30°C range there is little effect upon the rate of Photosynthesis. Temperatures abcve 30°C actually resulted in a rapid decline in photosynthesis. With these relationships between production and consumption of photosynthesis, it would be possible for respiration to consume more carbohydrates than were being produced. Werner (43) shows that at lower temperatures, around 20°C, the greatest portion of photosynthate was available and utilized in tissue assimulation or accumulated as storage compounds. Nightingale (24) grew tomatoes at

various continuous temperatures and showed that tissue development and storage of carbohydrates increased up to a temperature of 70°F. Above this temperature, tomato plants failed to store carbohydrates or to consistantly assimulate tissue. Above 95°F these failures brought about death of the plant.

Temperatures also effect nutrient uptake, because salt accumulation is highly dependent upon root cell respiration, which, in turn, is greatly effected by temperature. This has been confirmed experimentally by Hoagland (13) who showed that there was an indicated Q_{10} of the ion accumulation process of two to three between 10° and 30°C.

Relationships between tissue ion accumulation, carbohydrate levels, and growth by Graber (8, 9), Sullivan (33, 34), and Pinck (27) further indicate no one system should be studied separately from other systems.

Graber's and Ream's (8, 9) work on nitrogen levels and carbohydrates indicated a strong inverse relationship between the two. High levels of nitrogen fertilizer with high levels of leaf tissue nitrogen were accompanied by low concentrations of carbohydrates. Sullivan and Sprague (33, 34) showed a decrease in fructan with nitrogen fertilization. Pinck and Allison (27) showed high nitrogen levels created a condition where photosynthate was converted into leaf tissue rather than translocated into roots.

Similar work by McLean (22), Dexter and Johnson (7, 18), and Harrison (11, 12) showed that there was a decrease in root development under high nitrogen and an additional decrease by defoliation to the marked effect of death to such species as Agrostis capillaris.

Lawton and Shephard (19) have shown an inverse relationship between potassium and fructose in corn. Low levels of potassium were accompanied by high levels of fructose in the leaves. But there were marked reductions in root and ear development, apparently, because the plant was unable to mobilize and translocate these carbohydrates to the non-photosynthesizing organs. This was substanciated by Wall (40) who showed that potassium deficiencies were accompanied by inorganic nitrogen accumulations in the roots and ears, which indicated that carbohydrates were not available to be utilized in the Kreb's cycle.

Both quality and intensity of light effects the physiological functions of plant materials through the auxin, growth, and photosynthesizing systems. Under field conditions, work by Thomas and Hill (38) and Schneider and Childers (29) has shown that apparent photosynthesis is directly proportional to light intensity in vegetation such as apple and alfalfa foliage, because of mutual shading. But, in work with the individual tissues, peak

photosynthesis rates were reached at light intensities from one-fourth to one-third of full sunlight.

Low light intensities favor stomatal closure, which restricts the entrance of carbon dioxide, which further reduces photosynthesis. High intensities raise the leaf temperatures and they, in turn, directly increase respiration, as indicated by Weintraub and Johnston (42) and Shirley (30, 31).

Meyer and Anderson (20) state:

> "The dynamic condition of water in plant tissue is largely controlled by the opposing effects of the transpiration and absorption processes."

These are then inter-related to the other physiological functions, so that adequate soil moisture is not always an assurance of plant tissue hydration. Work by Hogan (10) indicates that plants can apparently obtain with equal facility a supply of water from soils from the point of field capacity to near the permanent wilting point. No measurable decrease in growth, photosynthesis, or respira- was observed until soil moisture passed the wilting point. However, Schneider and Childers (29) have shown that once plant wilting occures, a definite decrease in photosynthesis occures and normal rates will not be attained until leaves have been rewetted from two to seven days.

PROCEDURE PART I

LOCATION AND MATERIALS

The following greenhouse study was conducted in the controlled climate chambers located in the basement of the Plant and Soils laboratory on Purdue's campus. The work began on December 7, 1957 and was terminated on February 22, 1958, a total of 77 days.

The fall weather had been unusually mild, but the bentgrass sod was not actively growing. Six strips of dormant established common creeping bentgrass sod were taken from the practice putting green on Purdue's campus. Excess soil was removed from the roots until the sod pieces were about one-half inch in thickness. These pieces were placed in a greenhouse for a two day adjustment period.

The strips of sod were trimmed to fit into eighteen by nine inch flats which were filled with common, unwashed sand to a depth which allowed the soil's original surface to be flush with the top of the flat. This facilitated sample collecting and provided about six inches of sand in which new roots could develop.

MANAGEMENT

After two days, the flats were transferred to the controlled climate chambers. Two flats were placed in each of the three chambers which had maintained temperatures of 60, 70, and 80 degrees F. Each chamber had a vari-

ation of plus or minus two degrees.

The sod was placed under a sixteen-hour light period and an eight-hour dark period. The long day and short night photoperiods prevented flower initiation, which would alter normal metabolic procedures within the plant tissue. The light period began at 07:00 hours and ended at 23:00 during the experiment.

Light intensity at the sod surface was 1000 foot candles as measured with a direct reading Weston light meter. The intensity decreased to a low of 800 foot candles as the fluorescent tubes aged. The fluorescent light which is low in red and far-red light rays was supplemented with unfrosted incandescent bulbs. Light quality was not determined but was considered uniform throughout the temperature chambers.

The flats were watered daily with deionized water, until excess water drained from the bottom of the flat. Moisture was not considered a limiting factor, because temporary wilting was not observed on any of the sod pieces. The flats were watered twice a week, Monday and Thursday, with Withrow's A nutrient solution. The solution was applied in the same manner as the water, until moisture drained from the bottom of the flats. This was the only fertilization that the flats received.

No observed disease or insect troubles were encoun-

tered during the entire experiment.

SAMPLING

The leaf samples were taken by hand clippers which had a special pan attached to one blade to catch the leaf clippings. The grass was cut at a one-inch height above the soil surface.

The clippings were placed in a beaker and covered with a moist towel to prevent the tissues from losing moisture while three one-gram samples were being removed for fructose determinations. The remaining material was oven-dried at 170° F for twenty-four hours before the dry weights were recorded.

The samples were always taken at 13:00 hours on the specified days. This time was rigidly maintained because of daily fluctuations in fructose content of leaf tissue. Such diurnal changes in sugar content are brought about by production and storage of sugars during the light periods and mobilization and translocation of the sugars into the roots and stolens during the dark period.

A study of these daily variations of leaf fructose concentrations was made Fegruary 19 and 20, after the main study was completed. It was accomplished by taking samples of leaf tissue every four hours during the light periods, begining at the first hour of light.

Clippings were taken whenever the growth warranted

them until December 30. Then a schedule was established and observed until the end of the experiment.

Samples were taken on Tuesday, Thursday, and Sunday of each week. By alternating from one flat to the other at each temperature, sufficient time was allowed for regrowth. This allowed samples to be taken three times per week, and the changes in fructose concentrations to be recorded more closely.

ANALYTICAL METHODS

Fructose was selected as the indicator sugar for the bentgrass plant because of the work of DeCugnac (6), who classified the main storage compounds in grasses as either sucrose and starch, glucose polymers, or fructan, a fructose polymer. Bentgrasses are in the Graminee levulferes, or fructan storing group.

McDonald(21) explained that basically the fructan polymers are made up of numerous fructose molecules added to a sucrose molecule. In living tissue, concentrations of the different carbohydrates are constantly changing, as is true of any dynamic system with diurnal, as well as day to day, fluctuations. For fructose, these changes are schematically represented in Figure 1, a chart taken from M. R. Teel (35).

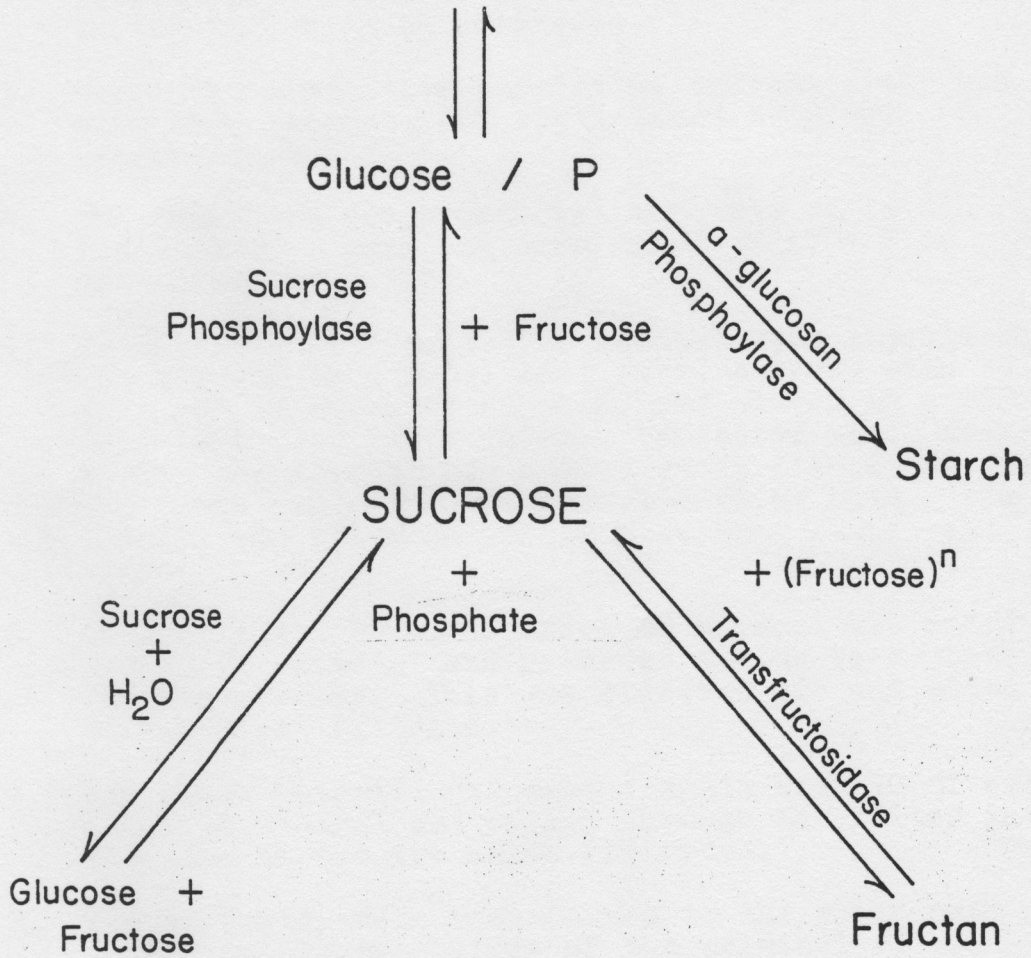
It was advantageous to determine not only the total concentrations of the storage compound, fructan, in relation

to the concentrations found in sucrose and as free fructose in the tissue. Whistler (44) has shown that it is possible to separate different lengths of carbohydrate polymers by extraction and using varying concentrations of ethyl alcohol and water. The polymers of longer chain length are more soluble as the concentration of water increases in the extraction solution. A preliminary extraction using absolute alcohol was used to remove the simple fructose and disaccharide sucrose from the tissue. A second extraction was made with water, which removed the less soluble fructan polymers.

The extracts were kept separate; then they were acid-hydrolized to break down the sucrose into glucose and fructose and to break down fructan into fructose and molecules. The concentrations of fructose were measured in each solution by the procedure employed by McRary and Slattery (23). By using modifications, the excess chlorophyll which had been extracted from the tissue in the primary alcohol extraction was removed.

Figure 1

Carbohydrate transformations in plants



LABORATORY PROCEDURES FOR FRUCTOSE AND FRUCTAN ANALYSIS

1. Clippings from growing grass at a one-inch height were taken at 1300 hours each day.

2. The clippings were placed in a beaker and covered with a moist towel to prevent excess loss of moisture during the weighing processes.

3. Three representative samples of approximately one gram were weighed, but significant to 0.000 (thousandths).

4. The remaining fresh material was weighed to 0.0 (tenths) of a gram and oven dried at 170° F to determine dry weight.

5. These three representative samples were immediately transferred to a 50 ml test tube, covered with 30 to 35 ml of absolute ethanol, and placed in a 90° C water bath for ten minutes. Excessive water absorption by the alcohol was prevented by lightly stoppering the tubes while they were in the water bath. The alcohol was filtered off in a Buckner funnel and saved.

6. An additional 30 ml of absolute ethanol was added to the plant material and returned to the water bath for ten minutes. This was filtered off and added to the first filtrate.

7. The plant material was washed again with 20 ml of absolute ethanol and passed through the filter in addition to the two above filtrates.

8. The filtrates were transferred to the water bath and approximately two-thirds of the original volume was evaporated. This solution contained all the mono and disaccarides from the plant material.

9. One-fourth teaspoon of activated carbon and 10 ml of H_2O were added to the solution and it was again filtered through a Buckner funnel and washed with 20 ml of water and 5 to 10 ml of absolute ethanol to wash the mono and disaccrides from the carbon.

10. The filtrate was transferred to a 100 ml volumetric flask, diluted to volume, and saved for the colorimetric test.

11.. The alcoholic extracted residue (from 7) was transferred to its original test tube and digested with 30 ml of water for 30 minutes in the 90° C water bath. It is filtered and washed with 30 ml more of water.

12. This filtrate was transferred to a 100 ml volumetric flask, diluted to volume, and saved for the colorimetric test.

COLORIMETRIC PROCEDURE

1. 1, 2, 3, 4, and 5 ml were delivered from volumetric flask to five 50 ml test tubes. 4, 3, 2, and 1 ml of H_2O were added respectively to the test tubes. This was used to determine which was in the range of the standard curve.

2. 15 ml of 30% HCl were added (one volume of water to 5 volumes of HCl adjusted to specific gravity of 1.145).

3. 5 ml of a 0.1% alcohol resoricinol solution was added. (one gram of resoricinol to one liter of 95% ethanol).

4. The solution was heated in a 80° C bath for twenty minutes and stirred one or twice to mix solutions.

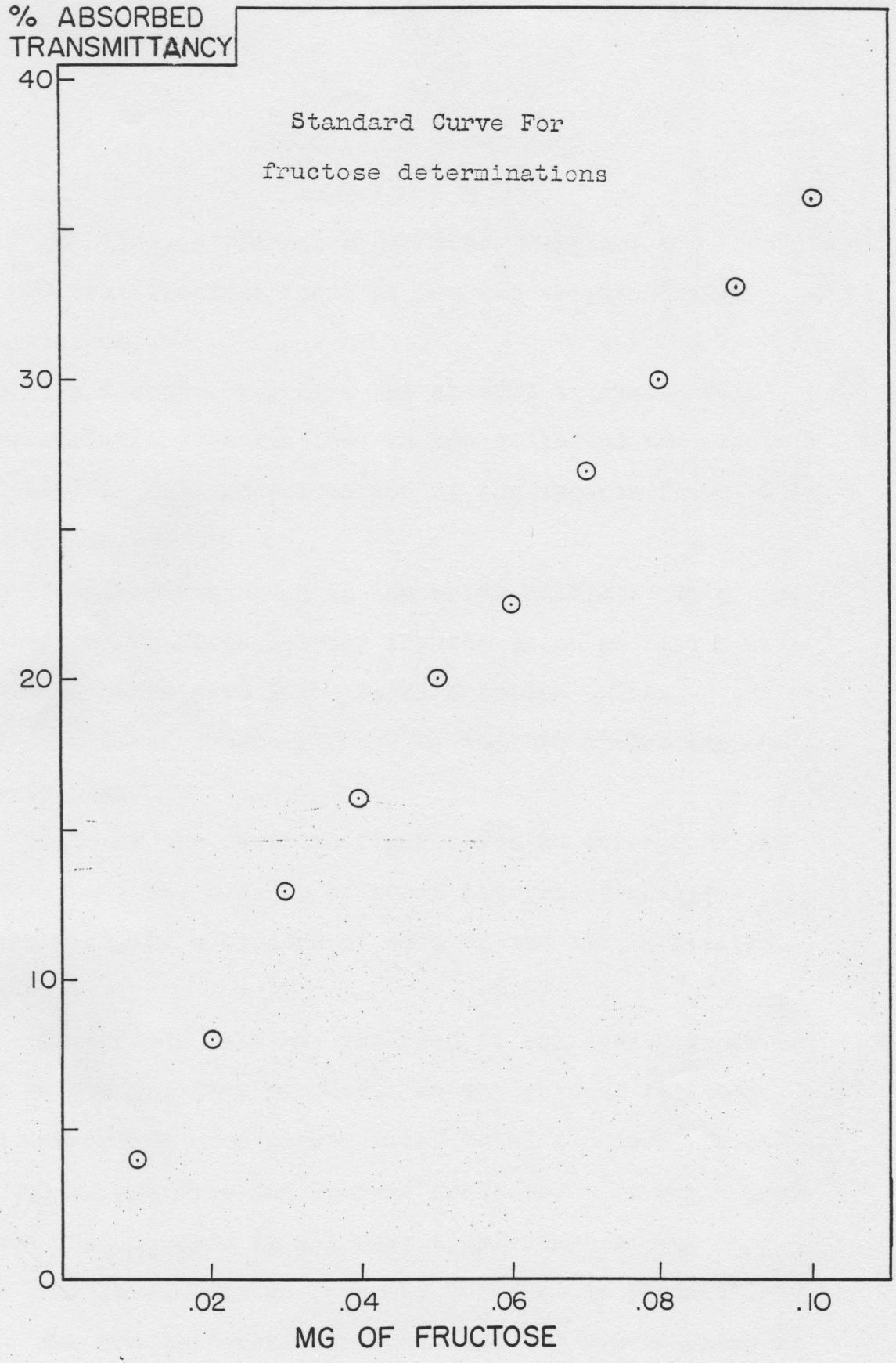
5. They were read in colorimeter, and 540 millimicron filter (green) was used.

6. The readings were compared against standard curve.

7. The per cent fructose was determined on a dry weight basis by the following procedure.

$$\% \text{ fructose of dry weight} = \frac{\text{chart reading in mg fructose}}{\text{ml used in color development}} \times 50$$

Figure 2


% ABSORBED TRANSMITTANCY
40
30
20
10
0
Standard Curve For
fructose determinations
.02
.04
.06
.08
.10
MG OF FRUCTOSE

RESULTS AND DISCUSSION

Temperature Study

The three different sugar determinations were recorded as per cent fructose found in the dry weight of the leaf tissues.

(1) The fructose found in the alcohol extract. This contained the free fructose in the cells and the fructose released by acid-hydrolization of the sucrose found in the tissue.

(2) The fructose found in the water extract. This contained the fructose polymer fructan which on acid-hydrolization broke down into simple fructose units.

(3) The total fructose found by the two preceeding extractions.

Each of the recorded figures was an average of six determinations, made up of three laboratory analysis taken from the clippings of each of the two replicate flats.

The growth rate was recorded as average in grams of dry weight per flat per day. An analysis of variance was determined upon growth rate, total fructose, fructan, fructose, and free and sucrose fructose. It was determined that changes in all were significant at the one per cent level between days and between the temperatures.

The fructose concentrations and the growth rates of leaf tissue grown at 60° F are given in Table 1; the graph

of these results is found in Figure 3.

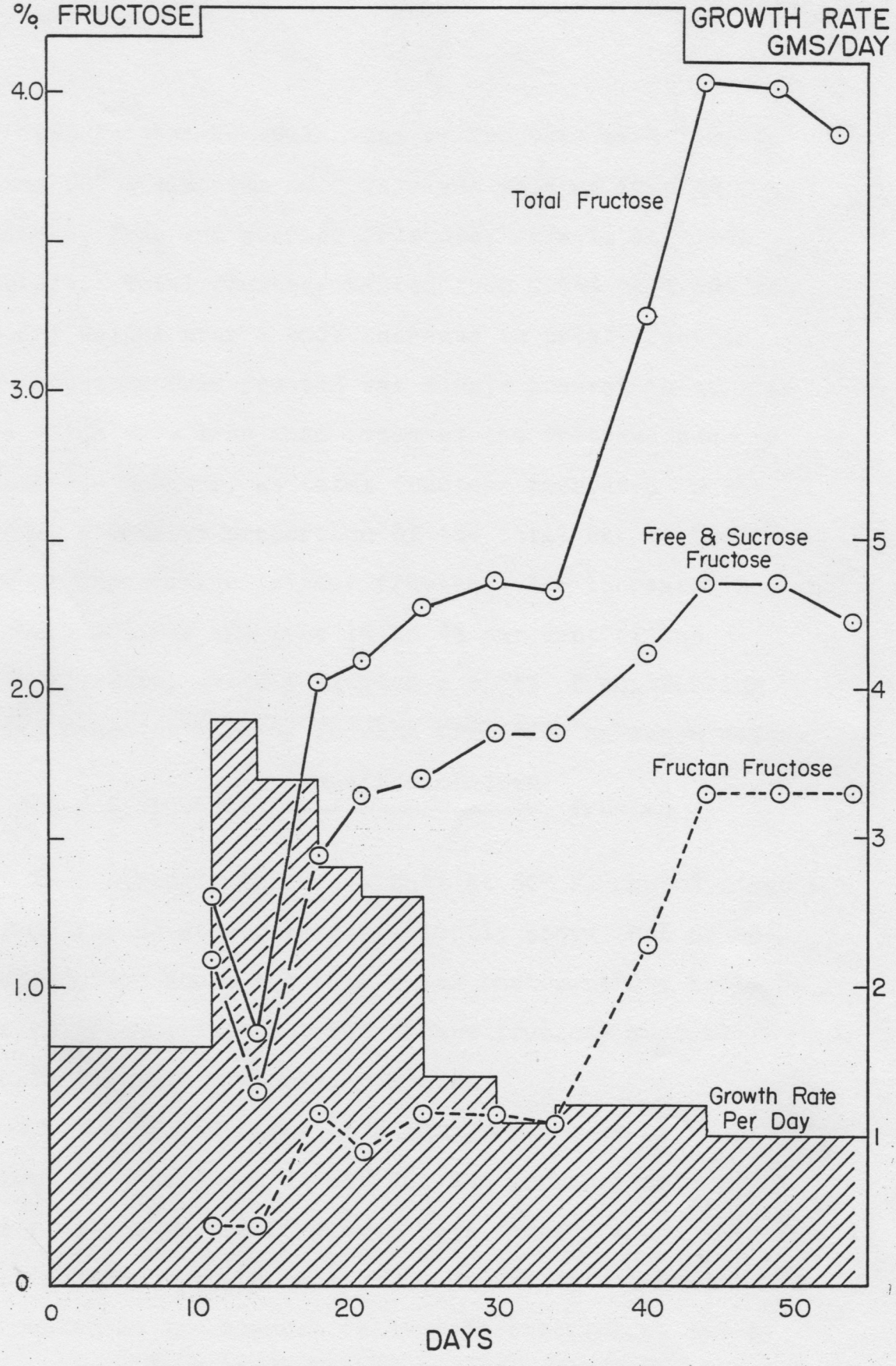
TABLE 1

Growth rates and per cent fructose as determined on the basis of dry weights of bentgrass leaf tissue grown at 60° F

	Fructan fructose	Free & sucrose fructose	Total fructose	Growth rate
Day	%	%	%	gms
11	.21	1.09	1.30	1.6
14	.19	.65	.84	3.9
18	.57	1.45	2.02	3.4
21	.45	1.64	2.09	2.8
25	.57	1.70	2.27	2.6
30	.56	1.80	2.36	1.4
35	.53	1.80	2.33	1.1
40	1.15	2.12	3.27	1.2
44	1.67	2.35	4.02	1.2
49	1.64	2.37	4.01	1.0
54	1.64	2.20	3.86	1.0
av	.84	1.75	2.58	1.72

Total growth = 180 gms

Figure 3



Average growth rates and fructose levels of leaf tissue grown at 60° F

The largest accumulations of fructose were found at the 60° F temperature. This was true of fructan fructose, free and sucrose fructose, as well as total fructose. Total fructose varied from 0.84% to 4.02% of the dry weight over a 400% increase in total fructose.

Fructose from fructan was always present in quantities which were less than those of the free and sucrose fructose. However, as total fructose increased in the tissue, a greater proportion of the total was in the form of the storage polymer fructan. The increase in fructan fructose was from 16 to 43 per cent of the total fructose, which indicated a shift of equilibrium in the reaction towards fructan formation as shown below.

$$\text{Sucrose} + n(\text{fructose}) \xleftarrow{\text{transfructosidose}} \text{Fructan}$$

This strongly indicates that at 60° F, photosynthesis is functioning at a rate sufficiently above that of respiration, and above the rate which photosynthate is being used in growth; so that the excess fructose must be transferred into a storage compound.

A total of 180 grams of clippings based on dry weights were produced at 60° F. The growth started very slowly with a period of eleven days needed before the first havest could be made. The growth rate increased and peaked at the highest daily rate observed at any of the temperatures between the 11th and 18th days. The [illegible] until the end of the experiment.

high rate of growth was short lived and rapidly declined until the end of the experiment.

A definite negative relationship existed between the growth rate and the levels of fructose found in the leaf tissues. This relationship is in agreement with McCarty (46), who established that carbohydrate accumulations were inversely proportional to the rate of vegetative growth. The magnitude of this relationship is shown with the simple correlation and regression of fructose upon growth rate in Table 4.

The decline in growth rate can not be attributed to a lack of photosynthate, used for energy or material with which to build new tissue, because the concentrations of sugar increased as the growth rate declined. The enzymatic systems which assimulate these simple carbohydrates into the complex compound of new tissue seem to function at a progressively lower rate at the continuous 60° F temperature.

The growth rates and fructose levels for leaf tissue grown at 70° F are found in Table 2, and the graph of the tables are found in Figure 4.

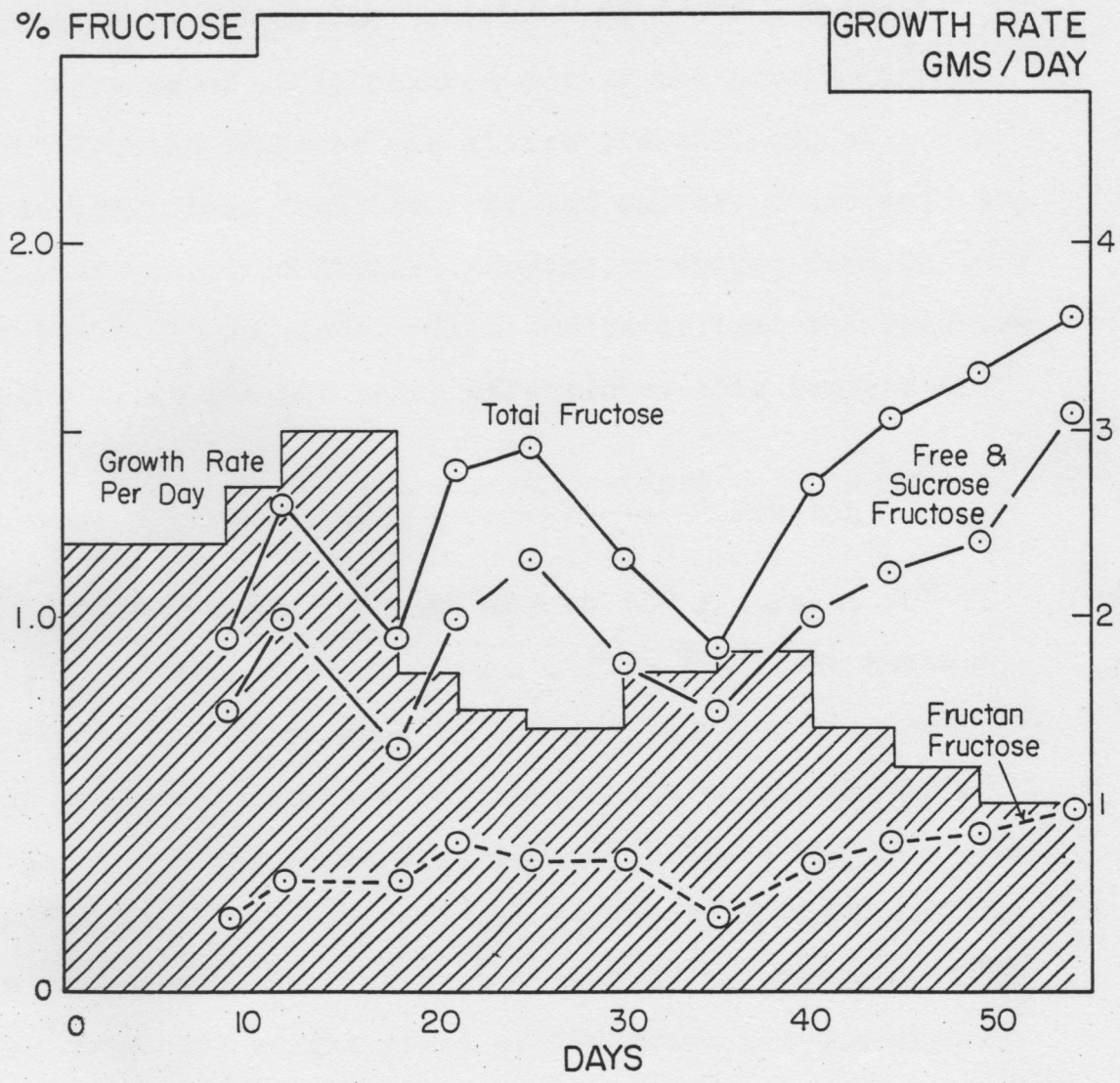
TABLE 2

Growth rates and per cent fructose as determined on the basis of dry weights of bentgrass leaf tissue grown at 70° F

	Fructan fructose	Free and sucrose fructose	Total fructose	Growth Rate
Day	%	%	%	gms.
9	0.21	0.75	0.96	2.4
12	.30	1.00	1.30	2.7
18	.30	.64	.94	3.0
21	.39	1.00	1.39	1.7
25	.29	1.17	1.46	1.5
30	.28	.86	1.14	1.4
35	.18	.74	.92	1.7
40	.35	1.01	1.36	1.8
44	.40	1.13	1.53	1.4
49	.42	1.21	1.63	1.2
54	.51	1.54	2.05	1.0
AV	.33	1.05	1.37	1.80

Total growth = 196 grams

Figure 4



Average growth rates and fructose levels

of leaf tissue grown at 70° F

The lowest levels of fructose, two per cent of the dry weights, were found at the 70° F temperature. An increase in concentration from 0.92 to 2.05 per cent, an increase of 200%, occured during the growth period.

Fructan fructose was always present, but at a concontration less than the free and sucrose fructose. The fructan exhibited little increase, a change from 22 to 25 % of the total fructose. This indicates that the reaction shown below was not being affected at this temperature.

$$\text{Sucrose} + n(\text{fructose}) \xleftrightarrow{\text{transfructosidose}} \text{Fructan}$$

Growth began more quickly at 70° F than at 60° F, requiring nine days of growth before the first harvest. There was no sharp peak in the growth rate, but there was a gradual increase in growth rate which reached a high of 3.0 grams per flat per day between the 12th and 18th days. This was followed by a very gradual decline in the growth rate during the rest of the experiment. The total dry weight yield of 196 grams was the highest yield of the three temperatures. This strongly indicates that the systems of photosynthesis, respiration, and growth are more in balance at 70° than at either of the two other temperatures. The fructose sugars produced were being used in growth of new tissue rather than being

accumulated in the leaf tissue. The smallest negative relationship is exhibited at 70° between fructose levels and growth, because the three systems are in better balance. This is shown in the simple correlation and regression of fructose upon growth rate in Table 4.

The changes in fructose levels and growth rates at 80° F are given in Table 3 with a graphic representation of the material in Figure.5.

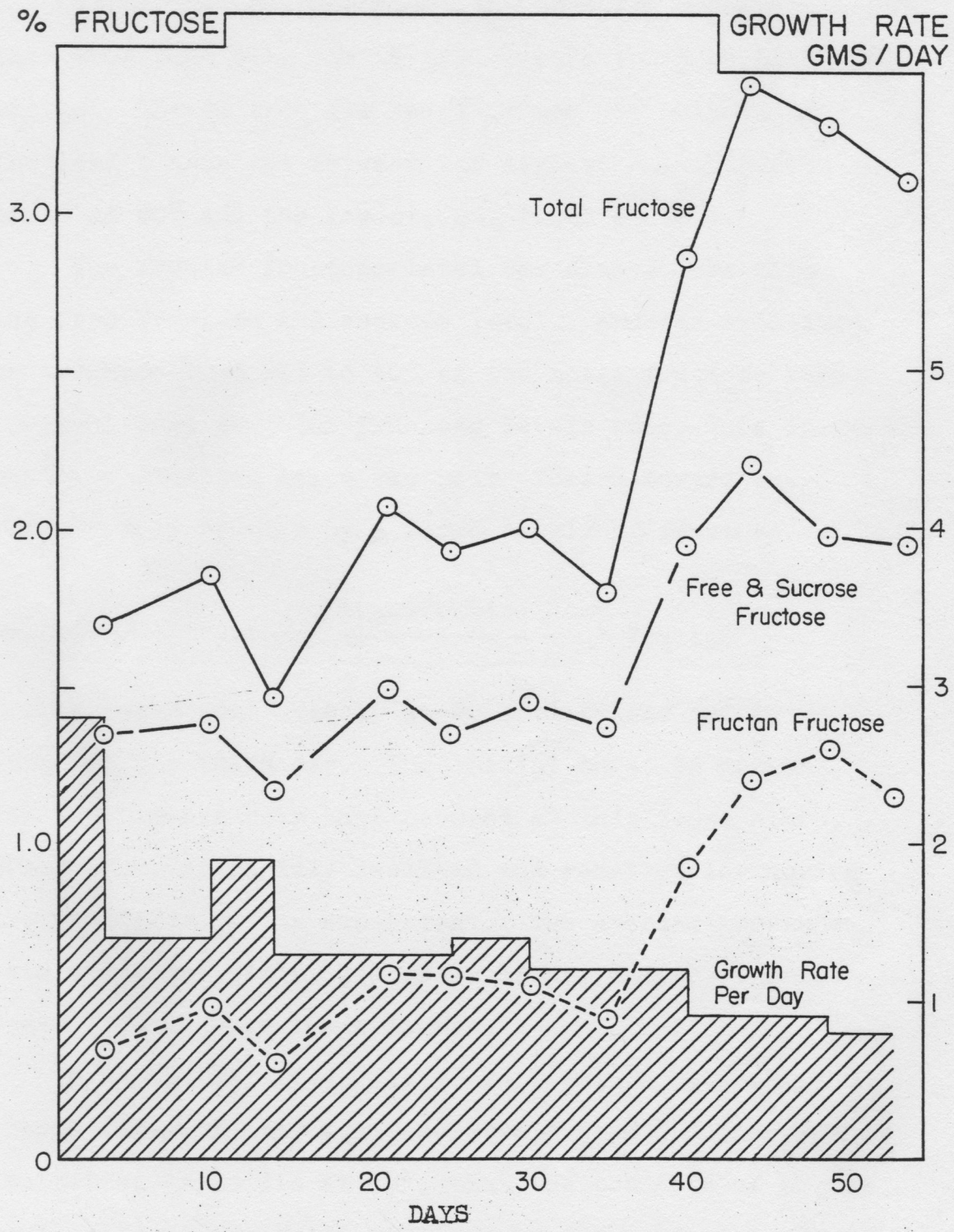
TABLE 3

Growth rates and per cent fructose, as determined on the basis of dry weights, of bentgrass leaf tissue grown at 80° F

Day	Fructan fructose	Free and sucrose fructose	Total fructose	Growth rate
	%	%	%	gms
3	0.33	1.35	1.68	2.8
10	.48	1.38	1.86	1.4
14	.30	1.17	1.47	1.9
21	.58	1.50	2.08	1.3
25	.58	1.35	1.93	1.3
30	.56	1.44	2.00	1.4
35	.44	1.37	1.81	1.2
40	.93	1.04	2.87	1.2
44	1.20	2.20	3.40	0.9
49	1.30	1.97	3.27	0.9
54	1.14	1.95	3.09	0.8
Av	.71	1.60	2.31	1.36

Total growth = 140 grams

Figure 5



Average growth rates and fructose levels of leaf tissue grown at 80° F

At the 80° F temperature the fructose concentrations increased 200% over the initial levels found in the leaf tissue. The 3% fructose level of the dry weights of the leaf tissue was between the highest concentration of 4% at 60° and the lowest, 2%, found at 70°.

The fructan fructose level was always less than the free fructose and sucrose level. Fructan exhibited an increase from 20% to 40% of the total fructose found in the leaves as total fructose levels rose. This inidcates that the reaction below was being forced toward the formation of fructan by a large supply of fructose.

$$\text{Sucrose} + n(\text{fructose}) \xleftrightarrow{\text{transfructosidose}} \text{Fructan}$$

Growth began most quickly at 80°; clippings had to be taken on the third day. The initial burst of growth was the highest rate that occured at this temperature. After which it rapidly declined and remained low during the remainder of the experiment. The average growth rate and total yield, 140 grams of dry matter, was the lowest of the three temperatures.

A strong negative relationship exists between growth rate and leaf fructose levels at 80°. This, again, indicates, as it did at 60°, that the products of photosynthesis are not being utilized in the production of new tissue as rapidly at they are being produced. The magnitude of this block is shown in Table 4.

TABLE 4

The simple linear regression and correlation of per cent fructose with a one gram increases in growth rate of bentgrass leaf tissue

	Coefficient of regression			Correlation Coefficients		
Source	60°	70°	80°	60°	70°	80°
Fructan fructose	-0.34	-0.06	-0.47	0.63**	0.52*	0.54**
Free and sucrose fructose	- .39	- .29	- .44	.74**	.66**	.57**
Total fructose	- .73	-.35	- .91	.70**	.67**	.61**

* Significant 5% level; r = 0.42

** Signigicant 1% level; r = 0.54

The regression figures in Table 4 give the predicted changes of per cent fructose levels for every one-gram increase in growth rate. Since this is an inverse function, every one-gram decrease in growth rate creates an expected increase in fructose. The magnitude of this increase is proportional to the numbers found in the table.

Figure 6 represents, in the form of a graph, the expected changes of total fructose percentages with one-gram changes in the growth rate.

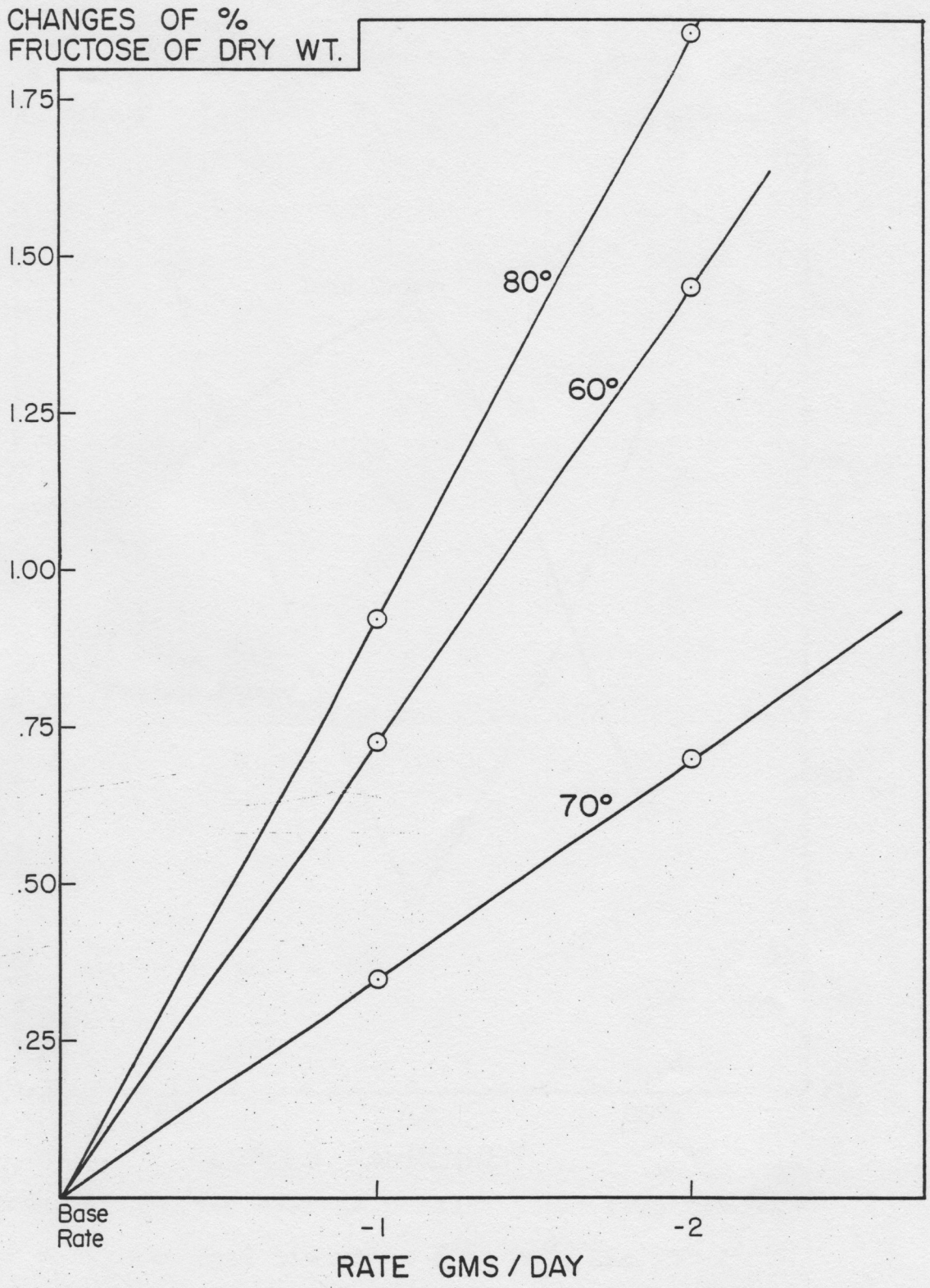
The results of Table 4 also indicate that in future work analyses of tissue for fructan fructose or total fructose should be used. They should be used, because of their greater range in variations at the respective temperatures.

(a) Fructan fructose had a range of 0.06 to 0.47, a eight fold variation.

(b) Total fructose had a range of 0.35 to 0.91, a 2.8 fold variation.

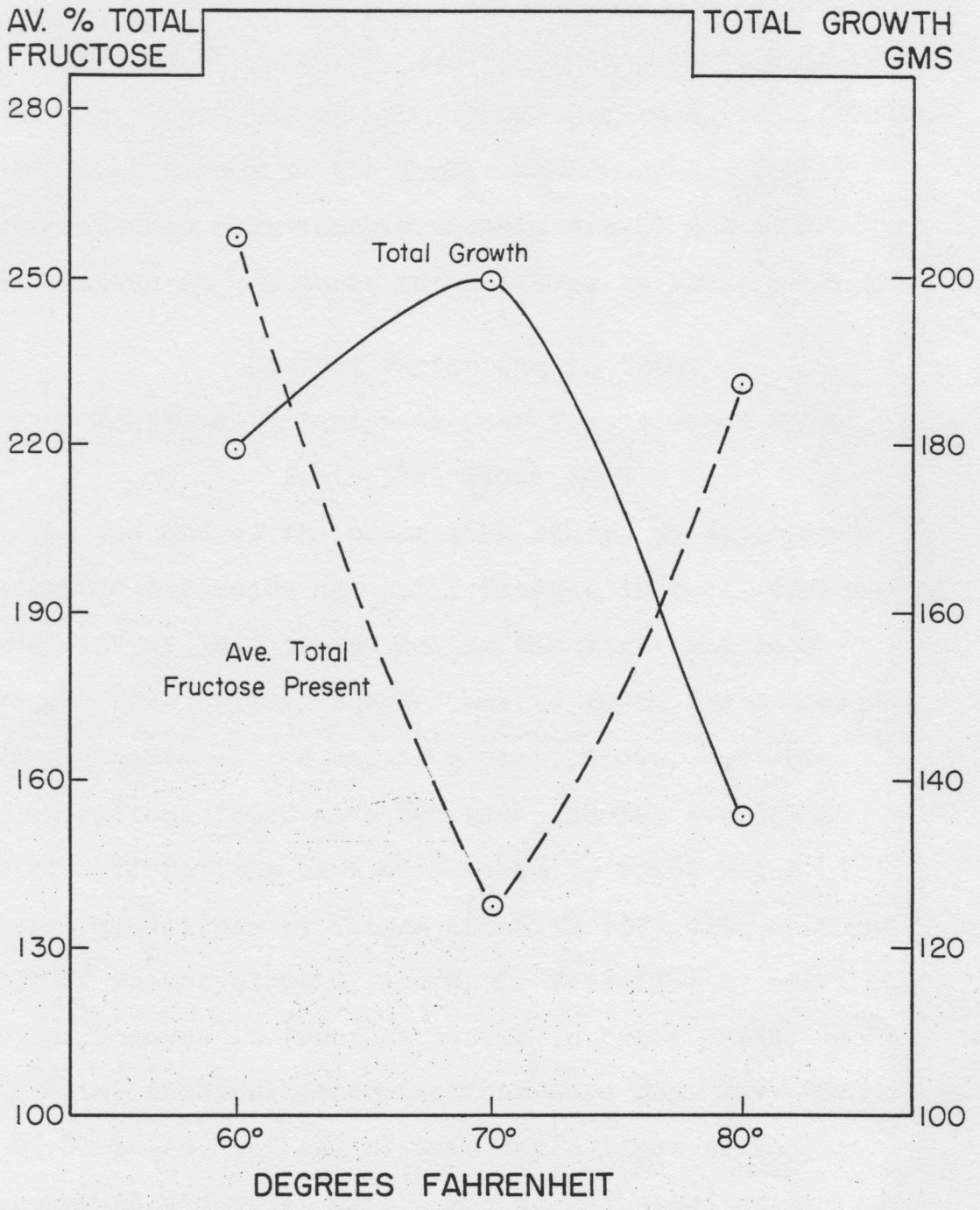
(c) Free and sucrose fructose had a range of 0.29 to 0.44, a 1.5 fold variation.

However, the fructan analysis is more troublesome to run when compared to the total fructose analysis. Therefore, future work will be based upon total fructose analysis rather than the fructan determination.



Prediction curves of changes of per cent total fructose for every one-gram decrease of the growth rate

Figure 7



Total growth and average percent total fructose of bentgrass leaf tissue at 60°, 70°, and 80° F

The summary graph, Figure 7, gives a comparison between total dry weight yields and average per cent total fructose in the leaf tissue at the three temperatures. The strong inverse relationship between growth and fructose accumulation at the three temperatures is clearly shown.

Diurnal Variations in Total Fructose in Bentgrass Leaf Tissue Grown Under Controlled Conditions

At the end of the preceeding study, an experiment was run to determine the daily changes in total fructose levels in the leaf tissue during the light and dark periods. The primary purpose was to check the concentration of sugars at the sampling hour, 13:00, against concentrations found at other times during the light period. Variations have been shown to exist under natural conditions by Thomas and Hill (37) with apparent rates of photosynthesis, and M. R. Teel (35) by measurement of changes in fructose levels in brome grass.

Total fructose determinations were run. They began at 23:00 hours, the end of that day's light period, on February 19. Samples were taken at the start of the light period, 07:00, the following day, February 20. Sampling was continued throughout the day at four hour intervals. Three lab analyses were run at each temperature for every sampling. The average total fructose levels by temperatures are given in Table 5.

TABLE 5

Diurnal fructose changes found in bentgrass leaf tissue

Time	60°	70°	80°
	%	%	%
Feb. 19, 23:00	6.0	4.8	4.9
Dark period	(no samples could be taken)		
Feb. 20, 07:00	3.8	1.4	1.6
" " 11:00	4.2	2.4	3.1
" " 15:00	5.0	3.6	5.7
" " 19:00	6.0	4.8	6.0
" " 23:00	5.6	4.4	5.4

Table 5 clearly indicates that the translocation system is functioning during the dark period and is removing fructose from the leaves. It also suggests that removal takes place at different rates at the respective temperatures. 13:00 hours may not be the best time for sample collection, but the time must be held constant before sampling in relation to the start of the photoperiod or in relation to the accumulated intensity hours of light.

PROCEDURE PART II

METHOD AND MATERIALS

The second phase of this study was conducted on the experimental putting green on the Purdue University campus from June 9 until October 1, 1958, a period of 115 days. During this time, data was collected on light intensities, soil moisture, and soil and air temperatures. A co-study was run by a fellow student, J. B. Beard, and his data on root numbers, depth, and color were used in the statistical comparisons.

Clippings of bentgrass leaves were taken at specific times during the experiment, dried, weighed and analyzed for total nitrogen, phosphorous, potassium, fructose, and reducing sugar.

The daily light data was taken on the green with a light integrater, a machine designed by Dr. A. J. Ohlrogge (26) of the Purdue agronomy department. Basically, the machine consisted of a photocell encased in an opaque glass globe, which directed all light received from various angles on to the photocell. When light intensity rose above 400 foot-candles, the cell developed a charge sufficiently large to activate a counter which then drained the charge from the cell. The speed at which this reaction took place was proportional to the light intensity within the 400 to 7000 foot-candle range. The accumulated counts per day ranged from 500 to 5000. These numbers had no specific value but gave a comparitive value of

daily light intensity conditions.

The moisture conditions of the rootzone were recorded at depths of 1,2,4, and 6 inches at two locations on the green. Readings were taken at 08:00, 12:00, and 18:00 hours and later averaged to give daily moisture conditions. The readings were taken on nylon Bouyocicous moisture blocks, covered with plaster of Paris, model CEL-WND3*. The range of readings was from 0.1 to 12.0 milliohms resistance or from complete saturation to approximately 20% available moisture.

Temperature recording equipment was set up and data taken by J. B. Beard (2). Temperatures were taken at 17 different locations every twenty minutes. The sensing elements were copper and copper constintane thermocouples attached to a speedomax multiple point recorder. Three points were located at 1, 12, and 36 inches above the green; two points were located in the mat of the green, and twelve points were located at 0.5, 1.5, 3, 6, 12, and 18 inches below the sod at two locations.

Root data was recorded by J. B. Beard (2) at specific times during the experiment. Root numbers were counted at 2, 5, 10, and 15 inches from cores taken on the green.

* Block and meter furnished by the courtesy of Industrial Instruments, Essex County, New Jersey.

There were four categories of root color: white, slightly brown, light brown, and brown. The percentages of the roots in each category present at 5, 10, and 15 inches were recorded in their respective color groups.

The bentgrass leaf clippings were repeatedly collected from 12 plots, each 3 feet by 48 feet in size, which were receiving variable nitrogen applications. The plots consisted of two replications of six treatments: heavy and light applications of urea, corn gluten, and uramite. The amount and number of applications were the variables, while turf quality and appearance were the constants. Other treatments such as disease and insect control were maintained constant throughout the plots.

The samples were collected with a 22 inch reel greenmower. first a border cut was made along the ends of each plot and the clippings discarded. Then a full length swath was taken out of the center of each plot from border cut to border cut, which collected a sample from approximately 80 sq ft. All of the sample was carefully transferred to a paper bag for drying, weighing, and analyzing.

The green was sampled 36 times during the experiment. Clippings were always collected before sunrise on the second day after a regular maintenance mowing. This standardized the regrowth period and alleviated diurnal

fluxuations in leaf fructose content.

Analytical procedures

Yield

The samples were placed in open paper bags and oven dried for two days at 170° F. The dry weights were taken and multiplied by the factor, 12.2, to convert to yield in grams per 1000 sq ft.

Fructose

Total fructose in all 432 samples was determined by a method adopted from McCrary and Slattery (23) which was very similar to the method used in the greenhouse study in Part I.

1. 250 mgm of dried plant material dried at 170°F, or lower, was weighed out and delivered to large test tube.
2. 15 ml H_2O was added, and it was heated at 90° C for 30 to 60 minutes. (Time was consistant.)
3. Extract was filtered and delivered into 100 ml volume flasks and deluted to volume.
4. 1, 2, 3, 4, and 5 ml of solution were delivered into color development tubes and 4, 3, and 2 ml of H_2O were added respectively.
5. 10 ml of 30% HCl was added to extract.
6. 5 ml of a 0.1% alcoholic resorcinal was added. (1 gm of resorcinal in 1 liter of ethanol.)
7. The solutions were placed in an 80° C water bath for 20 minutes.
8. They were read in colorimeter using 540 Mu (green) filter.

9. The readings were compared against a standard curve constructed with known amounts of fructose (.01 to .10 mgm) by the procedure used in steps 2 through 8.

10. Readings from standard curve were multiplied by 40 and equaled % fructose on dry weight basis.

Reducing Sugars

The total reducing sugars were determined for all of the samples The method used was adopted from Benham and Despaul (3) using aliquots from the water extraction of the fructose procedure. Readings were converted and recorded as per cent reducing sugars on the dry weight basis.

Reducing Sugar Determination

1. 10 ml of water extracted from 100 ml volumetric flask (from fructose procedure) was delivered to a colorimetric tube which contained the extract from 25 mgms of dry material.
2. 5 ml of 0.02 M potassium dihydrogen phosphate and 5 ml of 7.5% ammonium molybdate were added.
3. This was mixed and placed in a steam bath for 30 minutes (time was kept constant). The tops were covered to prevent steam contamination.
4. The solutions were read immediately in colorimeter using 660 M_u filter.
5. The readings were compared to the standard curve.
6. Per cent reducing sugars on a dry weight basis were determined by the following:

$$\text{Curve readings} \times 4$$

Standard Curve

1. Prepare fresh glucose solutions containing 5.0 to 50.0 mgms of sugar per 100 ml of solution.
2. Deliver 10 ml of each to colorimeter tubes.
3. Follow above procedure from step 2 and plot results.

Nitrogen

Total nitrogen was determined by a modification of a method developed by Umbreit, Burris, and Stahffer (39). Total phosphorous and potassium were determined by a procedure developed by M. R. Teel (36). Basically, the methods are micro-Kjeldahl techniques using a Nessler's colorimetric procedure which replaces the usual ammonia distillation and tritration. Aliquots of the digestion solutions were used in the phosphorous and potassium procedure.

1. The sample, 20 mgms of dried leaf material, was digested in a pyrex tube in a sand bath at 170° to 190° C with 1.0 ml of 5 N H_2SO_4 (containing 150 mgms of copper sulphate per liter) for 24 hours.
2. 30% H_2O_2 was added one drop at a time and heated in the samd bath until the solutions became clear.
3. The sample was cooled and diluted to 10 ml volume in the digestion tube.
4. 0.5 ml of digestion solution was transferred to a colorimeter tube. 1.4 ml of modified Nessler's reagent and 0.1 ml of 5.5 N KOH were added and volume was adjusted to 10 ml with water.
5. After ten minutes, it was read with a 520 Mu filter in a colorimeter.
6. The sample color was compared to a standard curve prepared with NH_2SO_4 in the range of 30 to 100 micrograms of nitrogen.
7. The readings were converted to per cent nitrogen by multiplying micrograms by the factor of 100.

Potassium Determinations

1. A 1.0 ml aliquot was taken from the 10 ml nitrogen digestion process, 9 ml of lithium nitrate solution (2.213 gms of lithium nitrate per liter) was added.

2. A Bechman flame photometer was used to determine parts per million of potassium in the solution. The internal lithium nitrate standards were used in all solutions.

3. The readings were converted to per cent elemental potassium by multiplying milligrams in the sample by 500.

Phosphorous Determinations

1. 1.0 ml aliquots were transferred to colormetric tubes from the nitrogen digestion process.

2. 6 ml of ammonium molybdate solution (63 ml of concentrated HCl and 5 gms of ammonium molybdate) were added.

3. 3 ml of elon solution (10 gms of elon in one liter of 3% $NaHSO_3$) were added.

4. The solutions were mixed and let stand 10 minutes.

5. They were read in colorimeter with a 660 Mu filter.

6. The solutions were compared to a standard curve based on p.p.m. of PO_4.

7. Reading was converted to milligrams and multiplied by factor of 50 to convert to per cent PO_4 on a dry weight basis.

RESULTS AND DISCUSSIONS

To determine whether the raw data* could be simplified for use in the multiple correlation calculations, an analysis of variance was run on the N, P, K, fructose, reducing sugar, and dry weight of clippings data. It was used to determine whether significant differences existed between the individual plots, treatments, replications, and sampling dates. The results are found in Table 6.

The F tests of the table indicate that within all parameters, a significant difference exists among sampling days. However, no significant differences were found among plots, treatments, and replications except in yield data and possibly one case in phosphorous data. By using this analysis it was possible to simplify the raw data to daily averages of the twelve plots in each of the six parameters.

* The data for individual readings are available on I B M cards from Dr. W. H. Daniel, agronomy department, Purdue University.

Table 6

Summary of F tests from the analysis of variance on nitrogen, phosphate, potassium, fructose, yield, and reducing sugar data.

	F test values					
Source	N	PO_4	K	Fructose	Reducing Sugars	Yield
Plots	1.9	3.3	2.3	2.5	1.0	9.2*
Treatments	3.0	6.2*	3.8	4.4	1.0	18.6**
Replications	.8	.6	1.6	.4	.6	3.4
Dates	24.9**	27.1**	30.5**	81.7**	19.6**	96.3**

* Significant 5% level

** Significant 1% level

Sixteen factors were selected from the 38 which were measured, and these were used in the multiple correlation analyses. They are listed and explained below.

X_1 Yield in grams of clippings per 1000 sq. ft.

X_2 The average per cent fructose (on a dry weight basis) of the collected clippings on that day.

X_3 The average daily per cent reducing sugars of the collected clippings on a dry weight basis.

X_4 The average daily per cent nitrogen of the collected clippings.

X_5 The average daily per cent phosphorous of the collected clippings.

X_6 The average daily per cent potassium of the collected clippings.

X_7 The average moisture condition on the day preceding clipping at a one inch depth, measured in milliohms resistance.

X_8 The average moisture condition on the day preceding clipping at a two inch depth, measured in milliohms resistance.

X_9 The light intensity number from the light integrater of the preceding day.

X_{10} The maximum temperature on the day preceding clipping at the 0.5 inch siol depth as measured in degrees F.

X_{11} The minimum temperature in degrees F on the day preceding clipping at the o.5 inch soil depth.

X_{12} The daily temperature in degrees F at the 0.5 inch depth on the day preceding clipping.

X_{13} The average daily temperature in degrees F at 36 inches above ground on the day preceding clipping.

X_{14} The root numbers found at the two inch depth on the day preceding clipping.

X_{15} The per cent of the total roots which were light brown in color at the five inch depth.

X_{16} The per cent of the total roots which were white in color at the five inch depth.

Data was collected on all 16 parameters 23 times during the experiment. This data is listed in Table 7*.

The analyses were run on an electrical digital computer, operated by the statistical laboratory at Purdue Uvinersity. The data using 15 independent and one dependent variables were run in a multiple linear regression model. These computations were necessary to develop a matrix which were used to select the multiple correlation coefficients (R^2). This term is an estimate of the per cent of the dependent variable which can be accounted for by one or by a combination of independent variables.

The technique used is the Wherry-Doolittle method which selects independent factors only when their addition creates an upward increase in R^2. This method selects the variable with the greatest correlation coefficient,

* The data are available on I B M cards from Dr. W. H. Daniel, Purdue University.

TABLE 7

Data for the multiple regressions and correlations

Day	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}
	gms	%	%	%	%	%	MO	MO	#	°F	°F	°F	°F	#	%	%
4	238	0.74	10.61	4.97	0.93	4.65	0.10	0.10	3167	89	67	76.4	76.0	275	20	00
11	129	.87	8.49	5.88	.74	4.73	.20	.20	1329	79	66	70.1	67.5	215	27	2
16	163	.62	10.18	5.05	.71	3.57	.40	.40	2755	85	64	72.7	70.3	180	31	4
21	157	1.03	11.08	5.72	.72	4.81	4.00	6.00	4599	89	67	77.7	78.6	275	33	6
25	154	.87	6.46	4.82	.82	3.23	.20	.20	3057	90	74	81.8	82.0	310	40	10
30	291	.92	10.87	5.93	.88	5.10	.30	.30	3038	97	71	81.0	78.4	165	54	3
35	325	.52	9.25	5.68	.85	5.64	.30	.20	3134	91	73	80.8	78.6	177	62	6
44	405	.80	8.90	6.02	1.01	4.75	.20	.20	1848	85	74	77.1	76.1	228	75	11
49	191	.52	9.17	6.71	.89	4.50	.20	.15	4244	93	74	82.5	82.3	275	46	5
53	215	.54	8.74	5.65	.88	4.79	.10	.10	2172	90	77	81.4	79.1	260	52	4
65	288	.39	9.45	5.68	.89	3.33	.20	.15	4531	99	77	86.2	84.7	122	66	10
70	59	.78	11.32	6.02	.69	4.30	.15	.15	3369	88	71	78.0	74.5	260	52	13
72	133	.61	12.01	5.01	.94	4.48	.40	.30	4094	88	68	77.5	75.0	280	46	16
77	125	1.16	11.32	5.30	.87	4.61	.10	.10	3477	86	70	77.3	73.7	360	38	23
84	186	1.09	12.28	5.66	1.16	4.54	.20	.15	4246	87	69	78.8	74.3	410	55	7
86	151	.60	12.01	6.09	.67	4.11	.20	.20	2824	85	68	75.8	75.3	418	54	7
88	139	.38	12.01	6.11	.83	3.63	.10	.10	3525	95	76	82.4	82.6	395	50	6
91	149	.76	11.01	5.35	.68	4.95	.60	.40	4165	87	69	76.4	73.0	391	44	4
95	238	2.00	11.17	5.45	.50	3.66	.20	.15	3456	78	62	69.3	64.8	340	36	9
100	493	1.42	6.99	5.88	.75	3.28	.10	.10	1485	83	73	77.0	75.7	220	40	2
102	243	1.71	10.22	5.81	.89	4.05	.15	.15	1944	78	64	70.2	66.3	180	44	1
107	164	2.01	10.89	5.49	.83	3.92	5.50	3.00	2658	81	63	72.4	74,7	256	44	1
114	88	1.39	10.39	5.38	.91	4.12	.10	.10	575	63	56	60.4	54.8	129	37	00
(av)	205	0.94	10.20	5.63	0.83	4.29	0.52	0.56	3030	86	69	76.6	74.7	270	45	7

conbines it with all other variables, and selects the pair with the greatest R^2 value. Then, combining this pair with all other variables, it selects the third variable which adds the greatest amount to the R^2 term until all variables are combined.

The multiple correlation results with yield as the dependent variable and the other 15 factors as independent parameters are found in Table 8.

Table 8

Multiple correlation coefficients squared (R^2) with yield as the dependent variable.

Independent variables	Predicted variations of yield
	%
Reducing sugars	24.1
Reducing sugars + % light brown roots	37.6
Reducing sugars + % light brown roots + fructose	42.7
Reducing sugars + % light brown roots + fructose + maximum soil temperature at 0.5 inch	47.9
Reducing sugars + % light brown roots + fructose + maximum soil temperature at 0.5 inch + moisture at 1 inch	52.5
All 15 independent variables	73.1

The R^2 values of Table 8 give an indication of what factors are important in predicting yield of clippings. This gives indications of which factors should be studied more closely and which factors could be abandoned in future work.

Using this model, reducing sugar has the greatest simple correlation with yield (-0.4907), which is significant at the 5% level. This indicates that a negative relationship exists between this component and yield, which is in agreement with the work by Sullivan and Sprague (33, 34) and Werner (43). If this paramenter were studied by itself, 24.1 % of the variations in yield could be accounted for.

The next most important parameter selected by the Wheery-Doolittle method (17) to be considered when combined with reducing sugars was per cent light brown roots at the 5 inch level. This parameter's correlation with yield is /0.3625. Although this figure is not significant at the 5% level, it indicates that the relationship is positive and that an increase in light brown roots causes an increase in yield. This is in agreement with Dexter (7), since browning of roots indicates a decrease in root vitality and should enhance top development. Using the two factors combined, 37.6 % of the variance in yield could be explianed. This is an increase

of 13.5% in variance.

The third parameter selected by the Wherry-Doolittle method was fructose, which has a simple correlation of 0.0782, which indicates almost no correlation. However, its direct influence and its interactions with reducing sugars and light brown roots increase the prediction of variation of yield to 42.7%, an increase of 5.1% over the first two. This indicates the importance of fructose in contributing to the physiological systems of growth as was indicated in Part I, the greenhouse study.

The fourth factor selected was maximum temperature at a 0.5 inch depth in the soil. Its simple correlation was 0.1845, which shows poor correlation with yield in this straight line model. However, the increase of predicting variations in yield was 5.2%, increasing the total to 47.8% for the combined four parameters.

The fifth factor selected was soil moisture at one inch depth. Its simple correlation was -0.1290, which again shows poor correlation. However, its simple effect upon yield enabled a 4.6% increase in the total 52.5% in the ability to predict variations in yield.

The computations of the Wherry-Doolittle process were stopped at this point, because of decreasing increments in predicting values and increasing costs involved in procuring the next parameter. A multiple correlation

coefficient was run for all 15 variables to determine the total amount of yield variation which could be predicted. Using all 15 parameters, 73.1% of the variations could be acccunted for.

A second similar multiple correlation study was run using the data from Table 9, but making fructose the variable, dependent upon the other 15 factors. This was run because the preceeding greenhouse work showed the importance of fructose in the physiological systems of bentgrass, and it appeared as one of the five parameters selected for predicting variations in yield in the preceeding work.

The same simple straight line model was used in this process as in the first, and the same statistics were calculated. The results from the multiple correlation computations are found in Table 9.

Using a straight line model and the Wherry-Doolittle method of selecting parameters, maximum soil temperature at 0.5 inches was selected as the greatest predicting variable. It has a simple correlation of -0.6518, which is significant at the 1% level. It indicates that a negative relationship exists between this variable and fructose levels of the clippings. This relationship is in agreement with work by Werner (43) and Nightingale (24) and others who have shown a strong inverse relationship

between temperature and photosynthate accumulations.

Table 9

Multiple correlation coefficients squared (R^2) with fructose as the dependent variable

Independent variables	Predicted variations of fructose
	%
Maximum soil temperature 0.5"	42.5
Maximum soil temp. 0.5" / soil moisture at 1"	57.8
Maximum soil temp. 0.5" / soil moisture at 1" / yield	63.9
Maximum soil temp. 0.5" / soil moisture at 1" / yield / light intensity	68.6
Mamimum soil temp. 0.5" / soil moisture at 1" / yield / light intensity / white roots at 5 inches	70.7
All 15 independent variables	88.6

If this parameter were studied by itself, 42.5% of the variations in fructose levels could be accounted

for.

The next selected parameter combined with maximum soil temperature, was soil moisture at the one inch depth. The simple correlation between it and fructose was 0.4580, which is significant at the 5% level and indicates a positive relationship with fructose. This could be explained because high moisture conditions are usually accompanied by lower daily temperatures and lower tissue temperatures.

The combining of the two parameters accounted for 57.8% of variance in fructose levels, an increase in per cent of 15.3. This increase can be attributed to the direct and indirect effects of moisture

The third parameter selected was yield which had a 0.0782 correlation which is very poor. However, when included, it enabled 63.9% of the variance in fructose to be accounted for, and increase in value of 7.1%. The large increase brings out the interrelation between carbohydrates and growth, which was demonstrated in the greenhouse work.

The fourth independent factor that was selected was light intensity — correlation with fructose was -0.3287 — which is not significant at the 5% level. However, it indicates that a negative relationship exists. This is feasible, since high light intensities are usually

accompanied by higher soil, air, and leaf temperatures, while maximum photosynthesis can be carried on at one-fourth to one-third of full sunlight. Therefore an increased light intensity over these values would have little effect on increasing levels of photosynthate. Adding light to the prediction equation of variations in fructose, increased the total by 4.7% to 68.6%.

The fifth factor selected was the per cent of white roots at the 5 inch level. The addition of this variable enabled 70.7% of the fructose variations to be accounted for, an increase of 2.1%. The selection process was stopped at this point, because additional parameters added such small increases to the total prediction value. A final calculation was run using all 15 variables to determine the total amount of variation which could be predicted.

SUMMARY AND CONCLUSIONS

Part I

1. Carbohydrate accumulation when measuerd by fructose accumulation is an inverse function of growth.

2. Fructan is the best carbohydrate indicator in bentgrass leaf tissue. Total fructose is the next best, and free and sucrose fructose is the least helpful when considered as a comparative indicator against growth.

3. Diurnal varriations of photosynthates do exist and are important considerations in timing of sample collection in a comparative study.

4. The growth systems of bentgrass leaf tissue are reduced at the constant 60° and 80° F temperatures. The systems of leaf growth seem to operate best and for longer periods of time at 70° F.

Part II

1. Variations in yield can best be explained by measurment of a limited number of physiological and ecological factors. As listed in order of descending importance, they are:

   (a) reducing sugars
   (b) % light brown roots
   (c) fructose
   (d) maximum soil temperatures
   (e) soil moisture

2. Variations in fructose can best be explained by measurement of these five factors. As listed in descending importance, they are:

   (a) macimum soil temperatures
   (b) soil moisture
   (c) yield
   (d) light intensity
   (e) % white roots

3. No one factor should be studied irrespective of all others that make up the total system, because every factor imparts direct as well as indirect effects upon every other component of a system.

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