ABSTRACT

SEASONAL RESPONSES OF SOLUBLE CARBOHYDRATES IN THE LEAVES OF FOUR COOL-SEASON GRASSES TO FIVE NITROGEN TREATMENTS

by David G. Green

The quantitative effects of applying 0, 3, 6, 9 and 12 pounds of actual nitrogen per thousand square feet on the soluble sugars in the leaves of Agrostis palustris, Festuca rubra, Lolium perenne and Poa pratensis were studied to determine the degree of carbohydrate depletion at higher nitrogen treatments. The mono-, di-, and oliogosaccharides were extracted with boiling 80 percent ethanol, separated via paper chromatograms, and the resulting sugar spots evaluated quantitatively with a densitometer. The polysaccharide fraction was observed to be a glucopolyfructan, extracted with boiling water and quantified colorimetrically by a ketohexose test.

The greenhouse environment produced leaf tissue considerably lower in oligosaccharide than field samples. Effects attributable to nitrogen treatments were most prominent in the oligosaccharide fraction, particularly oligosaccharides other than sucrose. The di- and monosaccharides failed to produce concentration differentials directly attributable to the nitrogen treatments. Treatments providing more than one and one-half pounds of actual nitrogen per thousand square feet per application produced near identical carbohydrate responses. Regrowth in the dark estimates of food reserve agreed favourably with chemical determinations, particularly values for oligosaccharide minus the sucrose fraction. Temperatures in the 80 F range and higher appeared to exert an adverse effect on growth

and carbohydrate reserves of the grasses studied. The total carbohydrates did not appear to be present in concentrations which were inadequate for growth.

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By

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INTRODUCTION

One of the objectives of nitrogen fertilization of grasses is to maintain plant growth. This growth is a result of the interaction between nitrogen taken up from the soil and reserve food materials already available to the plant. The utilization of nutrients, primarily nitrogen, absorbed from the soil solution requires metabolic energy and a supply of carbon skeletons, specifically organic acids. The major storage forms of this energy and organic acids are the soluble sugars, which are referred to as the carbohydrate reserve, or more generally, reserve food material. The objectives of this study were; 1) to determine the nitrogen treatments at which the carbohydrate reserve in the leaves became a growth-limiting factor, and 2) to determine the quantitative effects of various nitrogen treatments on these individual leaf sugars under greenhouse and field environments.

REVIEW OF THE LITERATURE

The importance of the sugars and sugar polymers, which are referred to as reserve or storage carbohydrates, to the utilization of available nitrogen by the plant is most easily elucidated by consulting a general textbook of biochemistry. Carbohydrates can be broken down via the glycolytic (Embden, Myerhof, Parnas) pathway and the citric acid (Krebs) cycle to various organic acids such as pyruvic, α-ketoglutaric, fumaric and oxaloacetic acids. These organic acids may combine by well established enzymatic reactions with ammonia, ATP and in the presence of magnesium ions form the corresponding amino acids. Chlorophyll synthesis from glycine and succinic acid is another reaction which uses organic acids. ATP, which stores the energy for these synthetic reactions, is also a product of this carbohydrate catabolism. Without either the carbohydrate to supply the energy and organic acids or a supply of available nitrogen, the plant is in an unfavorable position to carry out the synthesis of new materials.

Thomas (cited by Troughton, 23) defines a reserve food material as a substance that has a preliminary period of accumulation followed by a period in which the substance is maintained <u>in situ</u> at a relatively high concentration, and that later, in association with physiological processes taking place in the immediate vicinity or elsewhere, the concentration diminishes. According to Weinman (cited by Troughton, 23) certain groups of carbohydrates viz. sugars, fructosans, dextrins and starch are the most important reserve substances in grasses. Pentosans, hemicellusose and true cellulose were considered structural materials and not further utilizable as food reserve by the plant. McCarty, Brown, (both cited by Troughton, 23) and Sullivan and Sprague (22) hold similar opinions. The dominant form of reserve carbohydrate in cool season grasses according to De Cugnac's 1931 (4) classification are fructosans. Chemically the type of fructosan in grasses for the most part is the phlein structure, that is with a carbon 2, 6 linkage as contrasted to inulin's carbon 1 to carbon 2 linkage (9).

Archbold's (1) exhaustive review of fructosans in monocotyledons covers research prior to 1940. Troughton's (23) 1957 review of the underground organs of herbage grasses cites three workers, McCarty and Weinman, McIlvanie, and Aldous, who studied seasonal affects on food reserve material. They noted a decrease in concentration of reserve carbohydrates in the roots concurrent with early shoot growth and then a gradual increase during the late spring and summer followed by a decrease at the time of secondary herbage growth. McIlvanie associated maximum reducing sugars with rapid vegetative growth, maximum sucrose with differentiation and greatest quantities of "reserve polysaccharide" with the brief rest period prior to secondary growth.

These three authors plus Bews and Bayer, (23) and Arber (23) all noted carbohydrates are accumulated in the roots during the autumn.

Sprague and Sullivan (21) noted in orchard grass that high-nitrogen fertilization tended to reduce the percentage of fructan and low-nitrogen fertilization tended to increase it. They also noted that the stubble and lower 2/3 of leaf blades contained the highest weights of fructan.

The roots and upper 1/3 of the leaves were much lower in fructan. Sucrose was highest in the upper 1/3 of the leaves, and next highest in the roots. An inverse sucrose-fructan relationship existed. Sullivan and Sprague (22) also noted the regularity of loss of fructosan with temperature increase was similar in both stubble and roots.

Baker (23) working with Lolium perenne also observed that the percentage of total soluble carbohydrates was always greater in the stubble than in leaves or roots. Ehara and Tanaka (6) noted the fructosan contents of each organ of Italian ryegrass, Timothy, Bermuda grass and Bahia grass decreased as temperature rose, the concentration of fructosan in all four grasses being higher in stubble than in other plant organs. Hansen, et. al., (9) refer to Waite and Boyd's observation that the stems of growing plants commonly contain much higher levels of fructose polymers than do the leaves.

A direct method to measure organic food reserves in relation to growth of alfalfa was developed by Graber, et. al., (8). They transplanted alfalfa plants into pots which were placed in a dark growth room. Before and after dark regrowth values were recorded for dry matter, total sugar, dextrins and soluble carbohydrates, and total nitrogen. They concluded no very specific conclusions could be made from these studies per se, but that these procedures might be useful when interpreting results from chemical determinations for plant food reserves. Harrison, (10) and Juska (12) have used this method for evaluating bluegrass food reserves.

Jordan (11), used a modified version of the McRary and Slattery (18) Seliwanoff test for ketoses to study the effect of environmental factors on the carbohydrate reserves in the leaves of bentgrass (*Agrostis palustris*). He concluded fructan (phlein type fructosan) to be the best indicator of reserve carbohydrate in bentgrass, total fructose next in order of reliability, with free fructose and fructose from sucrose of least importance. A constant temperature of 70 degrees Farhenheit produced the optimal growth response with the corresponding negative effect on reserve carbohydrates.

The discovery of paper chromatography by Martin and Synge (16) and its subsequent application to carbohydrates provided a technique whereby the individual fractions of the carbohydrate reserve could be studied. Using these techniques as adapted by Laidlaw and Reid (13), Lopatecki *et. al.* (15) followed the quantitative changes of soluble carbohydrates in the stems of three wheat varieties during two growing seasons. They noted the presence of glucose, fructose, sucrose, oligosaccharide and fructosan (phlein type). The oligosaccharides (a number of low molecular weight monoglucopolyfructans) soluble in 80 percent ethanol were the dominant form of carbohydrate stored in the stems prior to ripening of the grain. Glucose showed maximum accumulation around heading and progressively decreased towards maturity. Fructose showed two peaks of accumulation, the first at heading and second during the dough stage. Sucrose concentration was appreciable at heading and gradually increased throughout the milk stage when monosaccharides were decreasing. Accumulation of fructosan in stems occurred after the initial build-up of sucrose. Oligosaccharide trends paralleled fructosan but in much greater quantities.

These references illustrate that the relationships between nitrogen availability and total soluble carbohydrate levels have long been established. They also indicate a lack of information regarding the effect of nitrogen on the carbohydrate fractions of which this soluble carbohydrate or food reserve is composed.

SECTION I

THE INFLUENCE OF NITROGEN TREATMENTS ON THE SOLUBLE CARBOHYDRATES IN LEAVES OF *LOLIUM PERENNE*, *AGROSTIS PALUSTRIS* AND *POA PRATENSIS* UNDER GREENHOUSE CONDITIONS

The objects of this experiment were; 1) to develop a suitable technique for analyzing large numbers of samples for soluble carbohydrates, and 2) to observe the quantitative effect of varying nitrogen treatments on these carbohydrates under greenhouse conditions

MATERIALS AND METHODS

Plant Culture Methods

Pure stands of bentgrass (*Agrostis palustris*, var. Toronto), bluegrass (*Poa pratensis*, var. Merion) and ryegrass (*Lolium perenne*, var. common) were planted October 15, 1962. The seeding rate used for the bluegrass and ryegrass provided 2,500,000 germinatable seeds per thousand square feet. The bentgrass was vegetatively planted. A randomized block experimental design was used with three replications. The flats were 8 inches deep by 9 inches by 24 inches containing a soil mixture of 50 percent sand, 40 percent loam soil and 10 percent vermiculite by volume. Approximately 4 pounds of 15-15-15 per thousand square feet (.6 pounds actual nitrogen, phosphorous and potassium) was mixed into the top 2 inches of soil in the flat prior to planting. The grasses were cut weekly to 1 inch height of cut with hand clippers. Water and fungicides (weekly application of Mildex from December 15 to May 27) were applied as needed. The plants received no artificial illumination during the course of the experiment.

No additional fertilizer was added to the treatment flats between October 15, 1962 and March 25, 1963. On March 25 a complete harvest was taken for chemical analysis. Treatments totalling 0, 3, 6, 9, and 12 pounds of actual nitrogen per thousand square feet using ammonium nitrate dissolved in two litres of water as the nitrogenous material were then applied in one application. Subsequent harvests were taken April 6, April 13, May 1, and May 27.

Analytical Methods

The selection of a suitable analytical technique was governed by the number of samples to be analyzed and the degree of accuracy required for following seasonal variations in soluble carbohydrates in a realistic fashion. The analytical procedures employed by Lopatecki et. al. (15) were based largely upon methods previously reported by Laidlaw and Reid (13). Laidlaw and Reid's (14) extraction and elution technique was combined with Dubois et. al. (5) phenol-sulphuric acid colorimetric determination and consistently accurate results were obtained. A more expedient method combined Laidlaw and Reid's extraction and separation on descending paper chromatograms with the utilization of a direct photometric reading as described by McFarren, Brand and Rutkowski (17), but without a recording integrator. This method eliminated the time required to locate and elute the sugars from the paper for colorimetric determination.

A procedure evaluation using D-xylose showed an average recovery for eight replications of 87.32 percent with a variation of 8 percent. These values were judged to be satisfactory for seasonal carbohydrate studies. A comparison of extraction methods using a Soxhlet apparatus versus extraction over a suction filter and Buchner vacuum flask produced results favoring the Buchner method (Appendix Table VI). The Soxhlet method, due to its reflux action, permitted the use of less solvent to obtain the same degree of extraction. However, the use of the Buchner arrangement required much less extraction time. By using a Flash Evaporator at reduced pressure and a temperature of 65 degrees C., the filter-flask extraction method was employed as subsequently described.

<u>Determination</u>

A. Samples

1) Hand clipped 10 grams fresh weight leaf tissue at 9 a.m. on clear days.

2) Immersed in 100 ml. boiling 80 percent ethanol for 5 minutes.

3) Stoppered and stored in refrigerator (temperature -9° F.).

B. Moisture Determination

1) Added approximately 2 grams fresh weight to a tared jar, and placed in oven at 105 degrees C. for 24 hours to determine percent moisture.

C. Extraction

1) Added 50 micrograms D-xylose to denatured sample (A-3) as a recovery check.

2) Macerated 10 gram sample for 3 minutes in a Waring Blender.

3) Alcohol fraction. Poured C-1 through Buchner funnel (1 sheet Whatman No. 2 filter paper) connected to a vacuum flask. When dry added additional 150 ml. boiling 80 percent ethanol. This

alcohol fraction contained mono-, di- and oligosaccharides. If tissue moistures within one grass variety varied more than 1 percent between treatments, calculated percent moisture and adjusted to a final 80 percent ethanol concentration in the filtrate.

4) Water fraction. Extracted with 150 ml. boiling water the filter residue from C-2. This water fraction contained the polysaccharides.

5) Made alcohol fraction to 250 ml., sampled 50 ml. aliquot and evaporated in Flash Evaporator at reduced pressure and 65 degrees C. to 10 ml. Similarly made water fraction to 150 ml., sampled 30 ml. aliquot and evaporated to 10 ml.

6) Hydrolysis. Divided alcohol fraction into two 5 ml. volumes. Made one 5 ml. volume 1 normal with HCL. Similarly divided 10 ml. water fraction into 2 equal volumes and hydrolyzed one fraction as above.

D. Separation

1) Prepared No. 1 Whatman (18-1/4 inch by 22-1/2 inch) as follows; drew lines at 1 inch, 2-1/2 inches, and 4 inches from top of paper. Labelled paper and spots (kept 1 inch minimum separation between spots).

2) Folded paper and placed hair drier (cold air) on opposite ends of origin line. Using lambda pipettes spotted 50 lambda volumes of alcohol and water fractions from C-6 to labelled origin. Similarly spotted standards (10, 50, and 100 micrograms glucose, fructose, sucrose, and D-xylose).

3) Clipped paper to antisiphon rod and lowered into dry solvent trough in Chromatocab. Placed support bar in trough. Added solvent

(7) to trough and removed antisiphon rod clips (solvent used; n-propanol, benzyl alcohol, 85 percent formic acid and water 50/72/20/20 v/v). Developed paper for 30 hours.

4) Removed paper by fastening clips to paper and antisiphon rod and placed in drying hood (room temperature) for one hour.

5) Sprayed with p-anisidine indicator (1 gram p-anisidine in 40 ml. n-butanol, plus 2 ml. 19 percent HCL). Dried 1 hour in hood.

6) Color was developed by placing paper 3-5 minutes in oven at 95-100 degrees C.

7) Scanned spots under Photovolt Densitometer (model 52-d) using a 445 mm. filter, and recorded maximum optical density. Constructed graph with optical density as the ordinate and concentration as the abscissa using readings from the standard spots. Converted optical density readings of unknowns directly to sugar weights per 50 microlitres.

E. Percent Dry Weight

$$\frac{\text{micrograms sugar}}{\text{10,000 times percent moisture}} \times 100 = \frac{\text{micrograms sugar}}{\text{moisture}} = \text{percent dry weight}$$

RESULTS

Table 1 represents the percent of dry weights for glucose, fructose, fructosan and sucrose occurring in Merion Kentucky bluegrass. The total carbohydrate values represent the summation of values for glucose, fructose, sucrose and fructosan. Sucrose and fructosan were the dominant carbohydrates in Merion leaf tissue under the greenhouse environment. Oligosaccharide was not present in leaf extracts during the greenhouse experiment. However, oligosaccharide was noted in material used in some preliminary investigations with leaves and stems. The initial effect of nitrogen fertilization applied March 25, 1963, was a lowering of all carbohydrate fractions to values below the 0 nitrogen (check) treatment. May 27, 1963, (63 days after nitrogen application) a separation between nitrogen treatments was noted. The lower the initial amount of nitrogen applied the higher was the resulting percent dry weight in terms of total carbohydrates. These results can be interpreted that as nitrogen becomes less available to the grass plants their rate of carbohydrate utilization and growth decrease, with the resulting increase in carbohydrates.

The values for sucrose did not differ too strikingly between the various nitrogen treatments. Little divergence from the 0 nitrogen treatment was evidenced. Sucrose appeared to be more affected by environmental factors other than nitrogen as evidenced by its decline between day 1 and 27 (other carbohydrate fractions were increasing in percent dry weight).

Table 1. Leaf carbohydrate percentages determined on a dry weight basis, occurring in Merion kentucky bluegrass under five nitrogen treatments. 1963.

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Fructosan	Total Carbohydrate
March 25	0	1.73	.27	.03	2.39	5.02
	3	1.95	.43	.44	1.97	4.75
	6	1.93	.20	.13	2.03	4.29
	9	2.27	.32	.68	1.47	4.73
	12	2.61	.47	.50	1.44	5.01
April 6	0	-	-	-	-	-
	3	1.49	tr.	tr.	tr.	1.49
	6	1.37	tr.	tr.	.29	1.66
	9	1.45	tr.	tr.	.37	1.82
	12	1.46	tr.	tr.	tr.	1.45
April 13	0	-	-	-	-	-
	3	1.93	tr.	-	tr.	1.93
	6	1.88	tr.	-	tr.	1.88
	9	2.20	tr.	-	tr.	2.20
	12	2.29	tr.	-	tr.	2.29
May 1	0	3.24	tr.	tr.	1.57	4.81
	3	3.24	tr.	.23	.80	4.27
	6	3.33	tr.	tr.	.33	3.66
	9	3.27	tr.	tr.	.25	3.52
	12	3.15	tr.	.23	tr.	3.38
May 27	0	1.91	.29	.40	1.04	3.64
	3	1.87	.15	.39	.53	2.94
	6	1.99	.20	.32	.33	2.84
	9	1.99	.31	.25	.17	2.72
	12	1.80	.20	tr.	.13	2.13

Soluble carbohydrate percent of dry weights for Toronto creeping bentgrass are in Table 2. Glucose and fructose levels were similar regardless of nitrogen availability. However, between May 1 and 27 fructose in the 0 nitrogen treatment showed a significant increase. Fructosan treatments showed an initially wide divergence on April 6, but by April 13 the 3, 6, 9, and 12 pound treatments had formed one group while the 0 treatment maintained a greater value. May 27 the 3 pound treatment also increased in value. The increase in fructose corresponds to the increase in fructosan, the fructose increase showing a lag in response relative to higher fructosan levels.

Sucrose, the dominant carbohydrate did not show a consistent response to the nitrogen treatments. As with the Merion kentucky bluegrass, sucrose trends in the bentgrass paralleled the check treatment.

Total carbohydrate with the exception of the April 6 harvest was highest in the 0 nitrogen treatment throughout the course of the experiment.

Traces of oligosaccharide were noted in all bentgrass plots prior to fertilization March 25. May 1 the 0 treatment and May 27 the 0 and 3 pound treatments contained oligosaccharide. Environmental factors in addition to applied nitrogen effected the oligosaccharide fraction. May 27 the 3 pound treatment again contained oligosaccharide, presumably an indication of depletion of available nitrogen. The disappearance of oligosaccharide in the 0 treatment on April 6 and 13 is attributed to environmental factors.

Table 3 represents soluble carbohydrates for common perennial ryegrass, under conditions identical to the Merion kentucky bluegrass and Toronto creeping bentgrass. No oligosaccharide was present in leaf extracts. Fructose and fructosan values were very low in all nitrogen treatments.

Table 2. Leaf carbohydrate percentages determined on a dry weight basis occurring in Toronto creeping bentgrass under five nitrogen treatments. 1963.

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Fructosan	Total Carbohydrate
March 25	0	1.61	.73	.30	1.44	4.18
	3	1.51	.80	.13	.85	3.29
	6	1.25	1.23	.19	.50	3.16
	9	1.88	.76	.19	.53	3.35
	12	1.10	.59	.35	.35	2.38
April 6	0	2.11	.23	.31	.93	3.48
	3	2.16	.23	.25	.81	3.45
	6	2.31	.34	.23	1.31	4.19
	9	1.88	.16	.23	1.63	3.89
	12	1.55	.11	.19	tr.	1.85
April 13	0	-	-	-	-	-
	3	1.13	tr.	.31	.43	1.93
	6	1.26	tr.	.34	.35	1.95
	9	1.85	.23	tr.	.26	2.34
	12	1.50	.13	tr.	.23	1.86
May 1	0	1.98	tr.	tr.	1.55	3.53
	3	1.83	tr.	tr.	tr.	1.83
	6	1.26	tr.	tr.	tr.	1.26
	9	1.80	tr.	tr.	tr.	1.80
	12	1.73	tr.	tr.	tr.	1.73
May 27	0	3.50	.19	1.25	1.64	6.43
	3	3.25	tr.	.13	.42	3.80
	6	2.64	.16	.15	tr.	2.95
	9	2.23	.21	.13	tr.	2.57
	12	2.85	.19	tr.	tr.	3.04

Table 3. Leaf carbohydrate percentages determined on a dry weight basis occurring in common perennial ryegrass under five nitrogen treatments. 1963.

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Fructosan	Total Carbohydrate
March 25	0	1.52	.54	.33	tr.	2.39
	3	1.43	.49	.29	tr.	2.21
	6	2.40	.42	.13	tr.	2.95
	9	2.40	.31	tr.	tr.	2.71
	12	2.29	.25	tr.	tr.	2.54
April 6	0	1.33	.34	.11	.45	2.23
	3	.87	.18	tr.	tr.	1.05
	6	.76	.06	tr.	tr.	.82
	9	.48	.05	tr.	tr.	.53
	12	.71	.11	tr.	tr.	.82
April 13	0	-	-	-	-	-
	3	2.07	tr.	-	-	2.07
	6	1.42	tr.	-	-	1.42
	9	2.07	tr.	-	-	2.07
	12	2.13	tr.	-	-	2.13
May 1	0	1.89	tr.	tr.	tr.	1.89
	3	1.60	tr.	-	-	1.60
	6	1.72	tr.	-	-	1.72
	9	1.72	tr.	-	-	1.72
	12	1.76	tr.	-	-	1.76
May 27	0	1.49	tr.	tr.	-	1.49
	3	1.37	tr.	tr.	-	1.37
	6	1.37	.27	tr.	-	1.64
	9	1.58	.36	tr.	-	1.94
	12	1.47	.13	tr.	-	1.60

The 0 treatment recorded slighly higher fructose and fructosan levels. Clucose values, while greater than fructose and fructosan did not illustrate a varying response to nitrogen. Sucrose was the dominant carbohydrate in ryegrass under greenhouse conditions. However, a poor correlation existed between soil nitrogen levels and carbohydrate values.

DISCUSSION

Total carbohydrate values for all nitrogen treatments generally were lower than the check treatment in all three grasses. No consistent differences between nitrogen treatments occurred (excluding check treatment). Sixty-three days after nitrogen application the 3 pound treatment values for total carbohydrate were intermediate between the 6, 9, and 12 grouping and 0 treatment for Toronto creeping bentgrass (Table 2). Nitrogen depletion in the 3 pound treatment is evidenced indirectly by this accumulation of carbohydrate. The single nitrogen applications appeared to provide excess nitrogen for plant uptake in all treatments and between nitrogen treatment (excluding the check), differences did not occur.

Sucrose was the dominant sugar in the three grasses (Tables 1, 2, and 3). Fructosan for Merion Kentucky bluegrass and Toronto creeping bentgrass were next highest (weight basis). These results are consistent with Sprague and Sullivan's (21) analysis of orchard grass (Dactylis glomerata). They observed sucrose to be approximately equal to fructosan in the upper two-thirds of leaf blades, but for all lower leaf and stubble parts fructosan was dominant over sucrose and the other sugars present. Considering the many different types of oligosaccharides present in plants, Hanson et. al. (9) point out that sucrose is the most important, both in quantity and in importance, to plant metabolism. There is evidence that sucrose is utilized in the formation of fructosan by transfructosylation. These considerations explain why in using leaf tissue, sucrose values greater than fructosan were obtained.

Jordan's studies based on leaf tissue of *Agostis palustris* indicated fructan to be a much better indicator of reserve carbohydrate in bentgrass than sucrose. Paech and Tracey (20) pointed out that the McRary and Slattery method for analysis of fructosans can be used only when 80 percent ethanol insoluble fructosans are present. Jordan used the above method on material extracted with water (90 degrees C. for 30 to 60 minutes). This extract would contain 80 percent ethanol soluble sugars. The possibility exists than Jordan's values for sucrose are low.

Oligosaccharides were noted in the Toronto creeping bentgrass on March 25, May 1 and May 27, and also in Merion bluegrass in preliminary investigations, but not between March 25 and May 27. No oligosaccharide and only low values were recorded for fructosan in ryegrass. Bacon (2) also reported the presence of fructosans in *Lolium perenne* but little oligosaccharide. He also noted raffinose to be present in green ryegrass leaves. Raffinose was not observed in any grass during greenhouse studies. The low values for oligosaccharide are attributed to environmental factors other than nitrogen treatments.

SECTION II

THE INFLUENCE OF NITROGEN TREATMENTS ON THE SOLUBLE CARBOHYDRATE FRACTIONS IN LEAVES OF AGROSTIS PALUSTRIS, FESTUCA RUBRA AND POA PRATENSIS UNDER FIELD CONDITIONS

The objectives of this experimental series were; 1) to improve the analytical methods used in section 1 for determining fructosan, 2) to observe the quantitative effect of varying nitrogen treatments on the soluble carbohydrates under environmental conditions, 3) to compare the addition of soluble nitrogen to turf in single seasonal applications versus fractional amounts distributed over a five-month period, and 4) to compare the technique of measuring plant food reserve as indicated by regrowth in the dark with carbohydrate reserve values determined by the chemical methods later described.

MATERIALS AND METHODS

Plant Culture Methods

These experiments were located at the Crop Science Farm, Michigan State University. Prior to 1961 the plot area was a low management grass sward. This turf was removed with a sod cutter and the soil augmented with course sand to produce a sandy loam soil to a six to eight inch depth. The area has a 1/200 foot slope which provides adequate surface drainage.

Toronto creeping bentgrass was dormant planted November 1961. Pennlawn creeping red fescue, Merion kentucky bluegrass and common kentucky bluegrass were seeded at 2,500,000 seeds per thousand square feet (seed and stolon source, Hiram F. Colwin and Sons, Detroit). The experimental design was a randomized block with three replications. Individual plots measured three feet by 25 feet. These areas received four pounds actual nitrogen prior to August 18, 1962.

Differential nitrogen treatments of 0, 3, 6, 9, and 12 pounds per thousand square feet per season of available nitrogen (ammonium nitrate carrier) were applied with a Scott's 3 foot spreader on August 18 and September 14, 1962, (each application 50 percent of seasonal total). The 1963 treatments were applied in one-sixth season's total nitrogen amounts April 18, May 15 and 31, June 15, July 16 and August 15. The ammonium nitrate was watered immediately after application to prevent foliar burn to the leaves. Analytical samples were harvested June 8 and 22, July 8 and 23, August 8 and 22, 1963. In addition, the Merion kentucky bluegrass was sampled May 11 and 23.

Similar methods were used to apply 0, 6, and 12 pounds per thousand square feet of actual nitrogen in single treatments August 5, 1963. No foliar burn occured. Samples were obtained August 5, 6, 8, 10, 12 and 16 and analyzed by the methods subsequently described.

The bluegrass and fescue areas were mowed at one and one-half inches twice weekly. The bentgrass was mowed four times per week at one quarter inch. Supplemental moisture was supplied by sprinkler irrigation. Other maintenance practices such as herbicide and fungicide treatments were applied as required.

Soil temperature data was provided by the Department of Agricultural Engineering's microclimate station located at the Horticulture Farm, Michigan State University.

Analytical Methods

A modified version of the analytical techniques described in Section I was used for all field samples. Experiments were conducted to determine the sugar moieties which composed the water fraction (polysaccharide) in the three grasses. Water fractions of Toronto creeping bentgrass and Merion kentucky bluegrass (Section I, analytical methods C-4) were applied in a 16 inch solid origin line to Whatman No. 1 chromatographic filter paper as described in Section I, analytical methods D. The paper was run for 133 hours using descending n-propanol, benzyl alcohol, 85 percent formic acid, and water (50/72/20/20 v/v) as the developing solvent. Marker strips 1 inch by 22-1/2 inches were developed with p-anisidine-HCL to locate non-migratory origin lines. The origins were eluted using the technique of Laidlaw and Reid (14), hydrolyzed with 1 N HCL and rechromatographed. Glucose and fructose standards were also included. The only sugar remaining after this processing was fructose. Glucose was not observed. Fructose was concluded to be the dominant sugar moiety in the water fraction. The McRary and Slattery (18) method for the colorimetric determination of fructosan was used to evaluate the water fraction. This modification eliminated the photometric determination of the hydrolyzed water fraction.

Determination

A. Samples

1) Sampled 10 grams fresh weight material at 10 a.m. on clear days. Harvested fescue and bluegrasses with a mower set to 1-1/2 inch height of cut; bentgrass was cut at 1/4 inch.

2) Denatured for 5 minutes in boiling 80 percent ethanol.

3) Stoppered and stored in refrigerator (minus 9 degrees F.).

B. Extraction

1) Added 2 microgram per 1 microlitre of D-xylose to A-3 as a recovery check.

2) Macerated A-3 for 3 minutes in a Waring Blendor.

3) Alcohol Fraction. Poured B-1 through Buchner funnel (1 sheet Whatman No. I filter paper) connected to a vacuum flask. When dry added 150 ml. boiling 80 percent ethanol. This fraction contained the mono-, di- and oligosaccharides.

4) Water fraction. Extracted (Buchner funnel, a second vacuum flask) residue from B-3 with 150 ml. boiling water. This fraction contained the polysaccharides.

5) The alcohol fraction was made to 250 ml., 100 ml. aliquot sampled and reduced under vacuum in a Flash Evaporator to 10 ml.

6) Hydrolysis. The 10 ml. alcohol fraction was equally divided.

7) One 5 ml. sample was made 1 N with HCL and left overnight at room temperature. Both the hydrolyzed and unhydrolyzed samples were spotted as in C.

C. Separation

Identical to Section I, analytical methods D, only 25 microlitres spotted (versus 50 microlitres in Section I).

D. Percent Dry Weight.

Identical to Section I, analytical methods E.

E. Water Fraction

1) .25, .5, 1, 2, 3, 4, and 5 ml. of water fraction B-4 were pipetted into 30 ml. test tubes and made to 5 ml. Prepared a

water blank.

2) Added 5 ml. 0.1 percent alcoholic resorcinol (1 gram resorcinol per 1 litre 95 percent ethanol).

3) Added 10 ml. 30 percent HCL (1 volume distilled water to 5 volumes of concentrated HCL). Mixed four times with stirring rod.

4) Placed in 80 degrees C. water bath for 20 minutes.

5) Poured into cuvettes and read optical density at 540 mu. (Bausch and Lomb Spectronic 20).

6) The readings were compared against a standard curve constructed with known amounts of fructose (10 to 100 micrograms fructose per 5 ml. water) by the procedure used in steps 2 to 5.

7) Percent dry weight.

$$\frac{\frac{\text{micrograms sugar}}{\text{ml. extract used}} \times 10^{-6}}{\text{total dry matter (grams)} / 25 \times 10^{-3} \text{ litres}} \times 100 = \text{percent by weight}$$

Methods for Measuring Regrowth in the Dark

The technique of measuring the amount of regrowth of plant materials in total darkness as an indication of organic food reserve was used to obtain data for comparison with results obtained by chemical analysis. Duplicate samples from all nitrogen treatments of bentgrass, Merion and common Kentucky bluegrass, and creeping red fescue were taken from the field and transplanted into pots. The potted grasses were clipped to within one-quarter inch of the crown prior to sampling, watered, and placed in a dark growth chamber at 70 degrees Fahrenheit, June 15, 1963. The plants were watered every second day and fungicide (Kromad) applied weekly. On July 11, after 26 days in the dark, the height in inches of regrowth one-quarter inch above the crown was recorded. This leaf material was subsequently removed, dried, and weighed.

RESULTS

Figures 1-A to 1-E illustrate seasonal levels of sucrose, glucose, fructose, oligosaccharide, fructosan and total carbohydrate in Merion Kentucky bluegrass. The oligosaccharide fraction contains sucrose plus lower chain glucopolyfructosans (identified by chromatographic separation, hydrolysis and a second chromatographic analysis) was estimated as the summation of each of the analyzed fractions, excluding sucrose as this value was included in the oligosaccharide fraction. All five nitrogen treatments failed to show consistent differences in their effect on the dry weights for sucrose, glucose, and fructose. 0 treatment fructosan values exceeded the 3 pound treatment. The 6, 9, and 12 treatments were the lowest. However, maximum values did not exceed one percent.

A more consistent trend occurred after June 8th in the oligosaccharide fraction. Higher oligosaccharide values were observed for the lower nitrogen treatments. Also, oligosaccharide was the dominant soluble carbohydrate in Merion Kentucky bluegrass leaves. The total carbohydrate levels after June 22nd were highest in the 0 treatment, followed by 3 and 6, with the 9 and 12 treatment levels the lowest.

Common Kentucky bluegrass carbohydrate fractions are depicted graphically in Figures 2-A and 2-B. No consistent carbohydrate trends relative to nitrogen treatments occurred. Seasonal responses were quite pronounced. The monosaccharide fractions decreased from early June until late August. Fructose showed a mild recovery August 8th and 22nd. Glucose remained in only trace quantities after July 27th. Sucrose and oligosaccharide values

Figure 1. Carbohydrate Concentrations in the Leaves of Merion Kentucky Bluegrass Relative to Five Nitrogen Treatments.

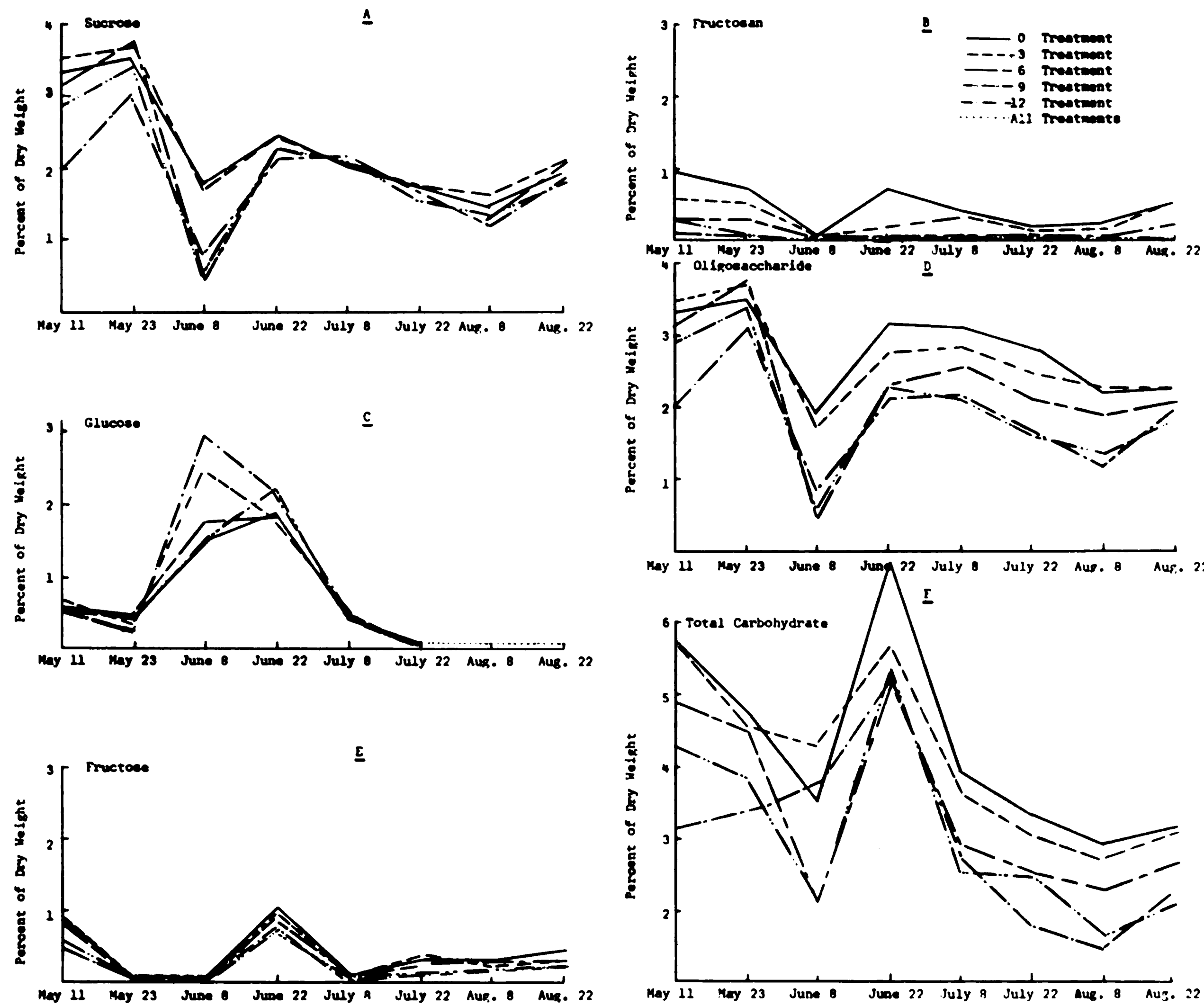
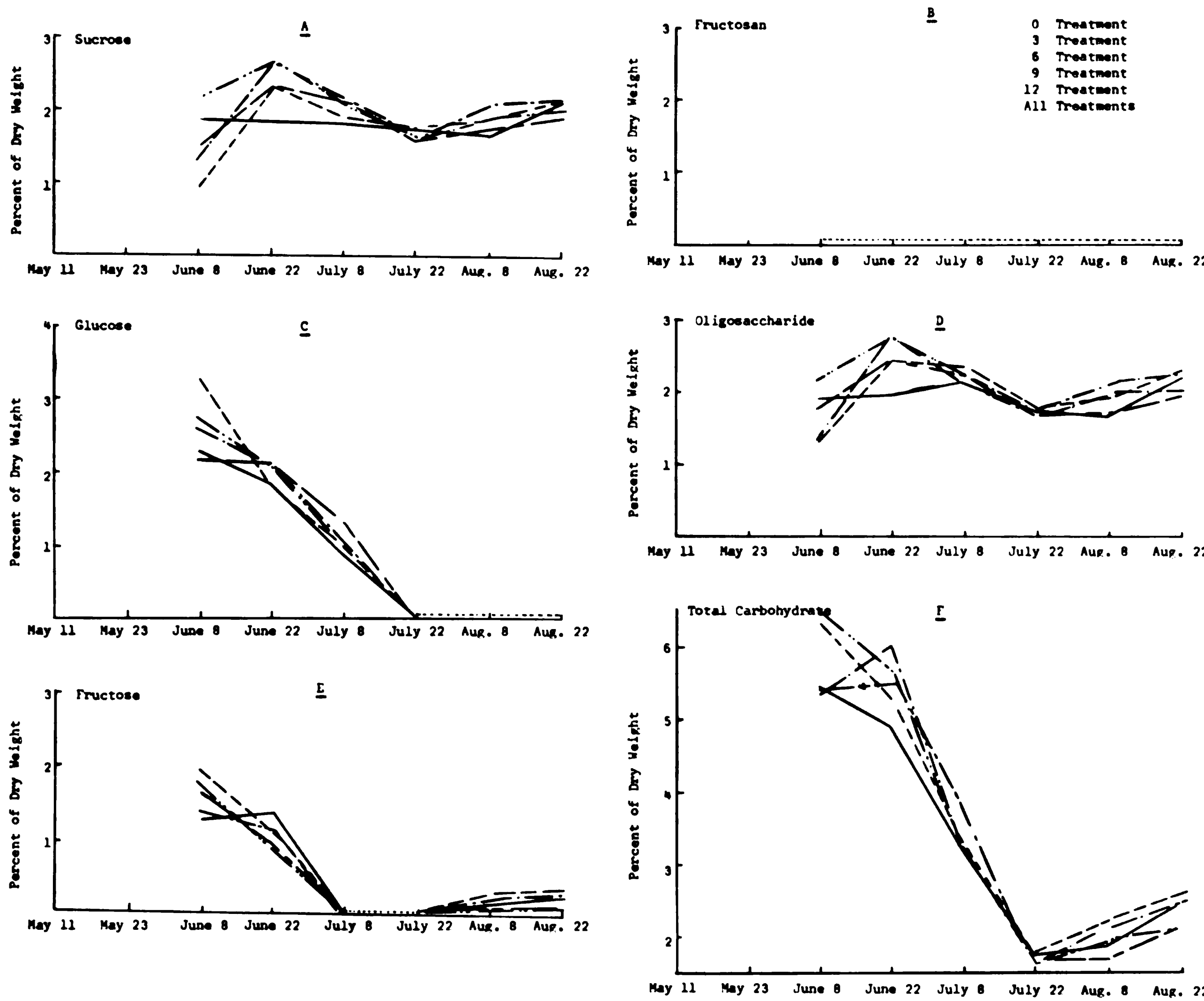


Figure 2. Carbohydrate Concentrations in the Leaves of Common Kentucky Bluegrass Relative to Five Nitrogen Treatments.



were nearly identical. This would indicate that sucrose almost exclusively made up the oligosaccharide fraction. Also, oligosaccharide values for June 8th to August 22nd deviated very little from the 2 percent level. Fructosan was present only in trace amounts. Total carbohydrate levels decreased very sharply from June 8 until July 22. This decline paralleled the depletion in the monosaccharide fractions.

Figures 3-A to 3-E illustrate dry weights for the soluble carbohydrates in Toronto creeping bentgrass. Glucose, fructose, and sucrose show definite seasonal variation, but lack differences attributable to the nitrogen applied. Only trace amounts of fructosan were present. Progressively higher oligosaccharide values resulted with decreasing nitrogen treatments. Total carbohydrate values showed two peaks of accumulation; the first in late June and the second in late August.

Sucrose, glucose and fructose concentrations in Pennlawn creeping red fescue (Figures 4-A, 4-C and 4-E) were independent of the nitrogen treatments. Sucrose values maintained approximately 1.5 percent dry weight throughout the summer season. Glucose and fructose decreased from a June 8 peak to trace values in mid-July. A slight recovery in fructose was noted in late August. Oligosaccharide (Figure 4-D) values showed a tendency to maintain values near 2 percent, but lacked a pattern related to the nitrogen treatments. Total carbohydrate values produced the most striking results. On May 23 the 0 treatment was 0.4 to 0.8 percent higher than the 3, 6, 9 and 12 treatments. On June 8 the 6, 9 and 12 treatments recorded a peak value (1 percent greater than the 0 and 3 treatments), and then declined until August 8. The 0 and 3 treatments showed a lag effect in

Figure 3. Carbohydrate Concentrations in the Leaves of Toronto Creeping Bentgrass Relative to Five Nitrogen Treatments.

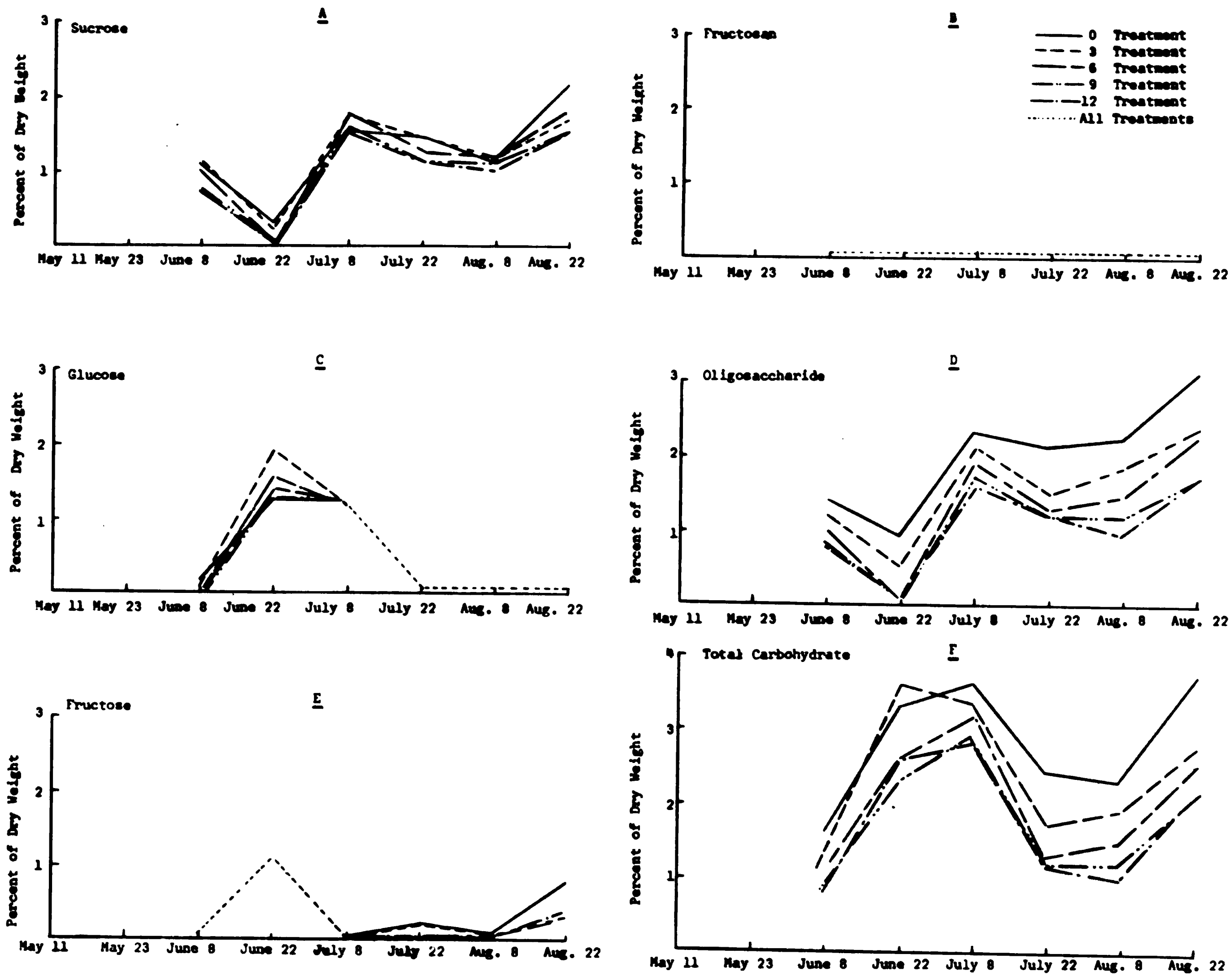
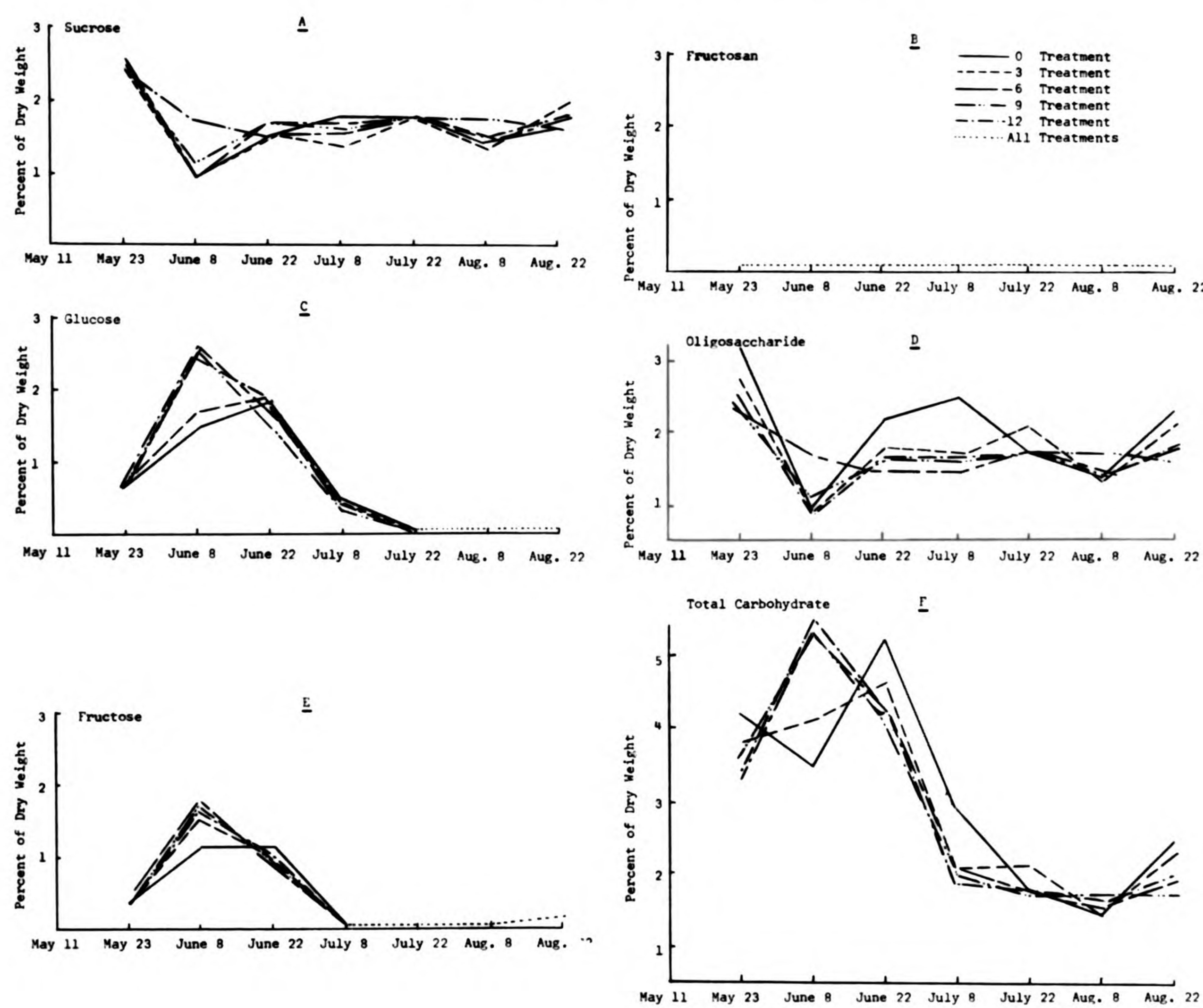


Figure 4. Carbohydrate Concentrations in the Leaves of Pennlawn Creeping Red Fescue Relative to Five Nitrogen Treatments.



reaching their peak concentration on June 22, after which they similarly declined. The 3 treatment was intermediate between the 0 and 6, 9 and 12 treatments June 8 and 22.

The results of applying 0, 6 and 12 pounds of actual nitrogen per thousand square feet per season in one application August 5, 1963, are illustrated (Appendix Table VII) for Merion kentucky bluegrass. In general, the 0 treatment values for all four carbohydrates were higher than the 6 and 12 treatments. The 12 treatment depressed the concentration of sucrose oligosaccharide and fructosan more rapidly than the 6. Seven days after nitrogen application, vegetative growth and carbohydrate responses were indistinguishable for the 6 and 12 treatments.

Common kentucky bluegrass (Appendix Table VIII) maintained relatively stable values for oligosaccharide (predominantly sucrose), irrespective of the nitrogen treatment. Fructosan values were less than 0.4 percent. Less growth response in terms of green colour and vegetative growth was observed for the common as contrasted to the Merion kentucky bluegrass. Merion contained both sucrose and longer chain oligosaccharides. Common kentucky bluegrass contained only the sucrose oligosaccharide August 5 to 16.

Sucrose concentrations for Toronto creeping bentgrass (Appendix Table IX) did not vary more than 0.4 percent between the three nitrogen treatments. The 0 treatment oligosaccharide concentration contained 1 percent more sugar three days after nitrogen application than did the 6 and 12 treatments. This large differential continued for the next nine days (experiment then terminated). The highest values observed for fructose and fructosan were 0.15 percent and 0.09 percent respectively.

Sucrose (Appendix Table X) was the predominant oligosaccharide in Pennlawn creeping red fescue August 5 to 16. No differential responses attributable to nitrogen treatments were observed. Glucose concentrations ranged from 0.2 percent to 1.5 percent with an over-all increase in values from August 5 to August 16. During this period, soil temperatures at the 3 inch depth decreased from 80 to 70 degrees Fahrenheit. No consistent nitrogen response was observed.

Results of the regrowth in the dark experiment (Appendix Table V) for the bluegrasses and bentgrass showed greater height and weight of regrowth occured in the lower nitrogen treatments.

DISCUSSION

Glucose, fructose and sucrose are not typical reserve food materials according to Thomas' definition. These sugars are active metabolically as intermediates between the initial products of photosynthesis and storage forms of carbohydrates, specifically oligo- and polysaccharides. Values for glucose, fructose and sucrose (Figures 1, 2, 3 and 4) for all four grasses studied under environmental conditions failed to exhibit characteristics directly attributable to the nitrogen treatments. Conversely, oligosaccharide, and fructosan when present, were in highest concentrations in treatments with the least nitrogen applied.

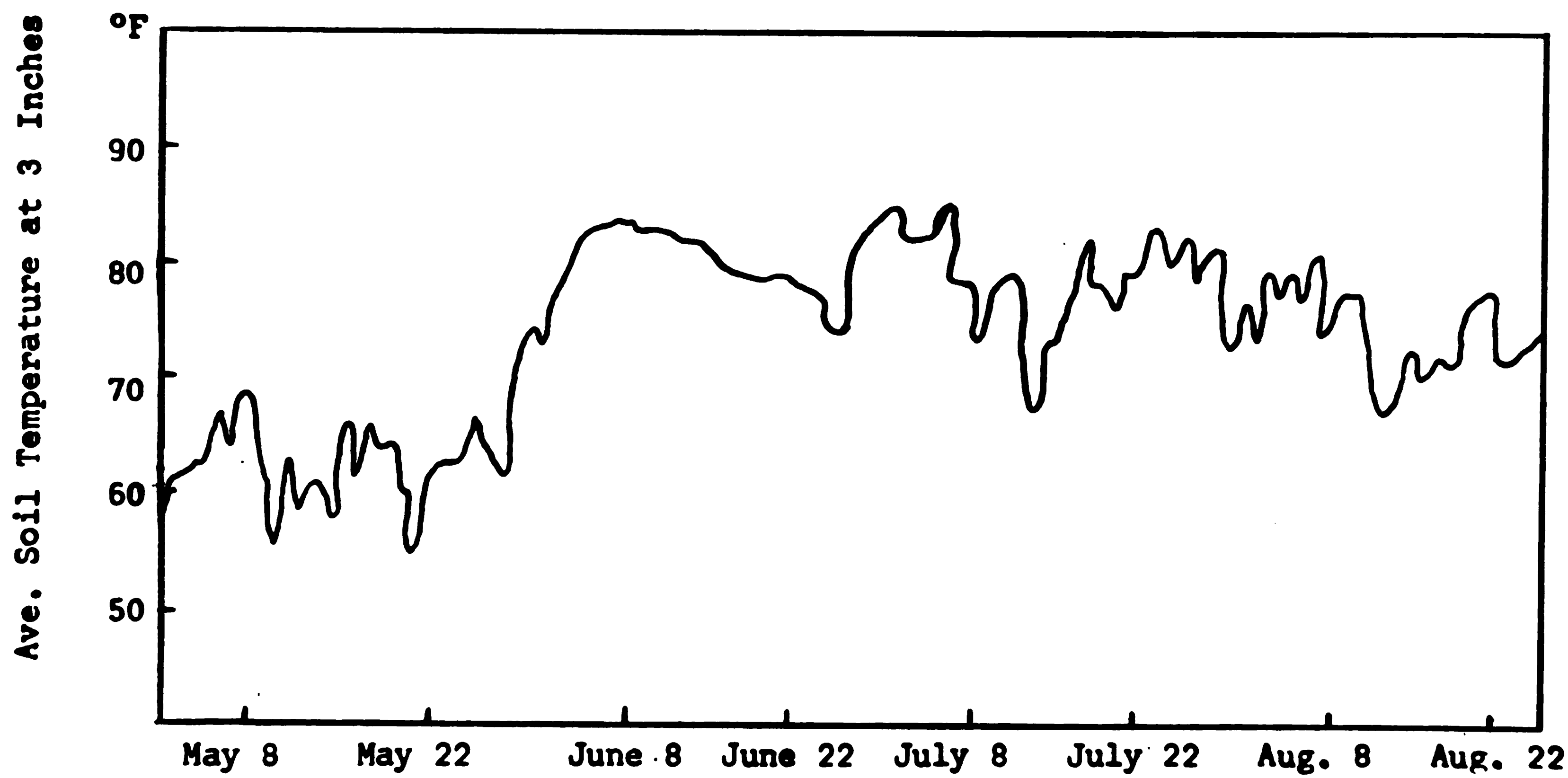
Leaf tissue, as Sprague and Sullivan (21), Baker (23), Ehara and Tanakea (6), Hansen et. al. (9), and others have noted, is lower in fructose polymers than is the stubble. This may account for the low oligosaccharide and fructosan values encountered. Leaves of Toronto creeping bentgrass and Merion kentucky bluegrass contained more sugar polymers than common kentucky bluegrass and Pennlawn creeping red fescue, and also produced more consistent carbohydrate responses attributable to nitrogen treatments. Merion kentucky bluegrass and Toronto creeping bentgrass responded faster in terms of vegetative growth to nitrogen than did common kentucky bluegrass and Pennlawn creeping red fescue. This possibly was due to the availability of leaf oligosaccharide. Carbohydrate differences between treatments during May for Merion and Pennlawn were negligible. Soil leaching and plant growth during autumn of 1962 nullified nitrogen differentials established in August and September. April 15, May 15 and May 31 treatments were necessary to restore these differentials. After June 8 oligosaccharide trends in Merion bluegrass and Toronto bentgrass were consistent, the low nitrogen treatments containing the highest concentrations of oligosaccharide.

The single nitrogen applications of 0, 6 and 12 pound treatments August 5, 1963, did not show any relationships between nitrogen treatments for grasses lacking oligosaccharide other than sucrose and fructosan. The 12 treatment oligosaccharide concentrations of bentgrass and Merion kentucky bluegrass, plus fructosan in the bluegrass dropped below the 0 treatment less than 24 hours after nitrogen application. The 6 treatment required more than 48 hours to produce similar results. No differences could be noted between the 6 and 12 treatments during the balance of the experiment. The tendency for the 6 and 12 treatments to parallel one another also occured with 3, 6, 9 and 12 treatments applied in one application as described in Section I. Nitrogen differentials for an experiment conducted over a period of several months should be applied in several applications to prevent the apparent excesses of nitrogen as observed in Section I and during the single-application field experiment. The grasses studied under the glasshouse environment lacked appreciable oligosaccharide in their leaves, hence no rapid carbohydrate-nitrogen interactions similar to those obtained in Merion and Toronto creeping bentgrass could be expected.

Jordan's study of <u>Agrostis palustris</u> under varying environmental conditions did not record the presence of oligosaccharides, other than sucrose. He concluded that fructan, the specific fructose polymer found in monocotyledons, was the best carbohydrate indicator in bentgrass leaf tissue. This was not the case under the environmental conditions encountered in this study. Oligosaccharide other than sucrose (Figure 5) was the most reliable indicator of differential carbohydrate reserves. Fructosan levels in leaf tissue were consistently low. Jordan's failure to note the oligosaccharide fraction is likely due to the inability of the method he employed to clearly distinguish the several sugars present in the alcohol extract.

Soil temperatures (Figure 6) at the three inch depth increased from 61 degrees F. May 22 to 83 degrees F. June 8. Sucrose values for Merion dropped from a mean of 3.48 percent on May 22 to 0.56 percent on June 8. The high nitrogen treatments showed the largest decrease, the 0 and 3 treatment combination dropping 1.86 percent versus the 9 and 12 combined decrease of 2.53 percent. Soil temperatures (Figure 6) remained in the high seventies and low eighties until August 12. Glucose and fructose values for Merion and Pennlawn, (Figures 1 and 4) in contrast to sucrose, increased in concentration with increased soil temperatures. After June 8 (June 22 for bentgrass) glucose and fructose in the bluegrasses and fescue decreased steadily until mid-August. Oligosaccharide regained values in late June and August which were considerably above the June 8 depression, and maintained these near constant levels regardless of the eighty degree soil temperatures. The advent of warmer soil temperatures (Figure 6) in early June produced responses in oligosaccharide similar to those caused by nitrogen fertilization. This initial heat effect appears to have diminished by late June and July as evidenced by a decrease in hexoses and increases in values for sugar polymers. Higher temperatures appeared

Figure 6. The Average Daily Soil Temperature at the 3-inch Depth May 1 to Aug. 31, 1963



to produce an adverse effect on percent total carbohydrate values for Merion bluegrass and Pennlawn fescue, with values decreasing from mid-June until mid-August.

Kentucky bluegrass and creeping red fescue are cool season grasses. Merion kentucky bluegrass and Toronto creeping bentgrass, while also cool season grasses, generally show less tendency to become dormant in hot weather. Lopatecki and McIlvanie both noted the highest hexose values to be associated with vegetative growth. Lower hexose values were noted during maturation, differentiation and dormancy. Late May and early June results with Merion and Pennlawn produced levels of hexoses which agreed with these worker's observations. All four grasses produced less vegetative material when 3 inch depth soil temperatures reached the high seventies. Merion and Toronto glucose and fructose values, while low, were higher than similar hexose values for common kentucky bluegrass and creeping red fescue during high temperature periods.

The total carbohydrate reserve generally includes hexoses, oligosaccharides, and polysaccharides. Earlier in this discussion the inclusion of hexoses in the reserve carbohydrate classification was questioned due to their active role metabollically. However, due to their tendency to be most prominent in concentration during periods of maximum vegetative growth and of lowest concentration during rest periods, a relationship which suggests a very direct association with sugar polymers, they represent an integral component of the total carbohydrate reserve.

Total carbohydrate reserve values recorded minimums with the 12 treatment in Merion kentucky bluegrass and Toronto creeping bentgrass of 1.43

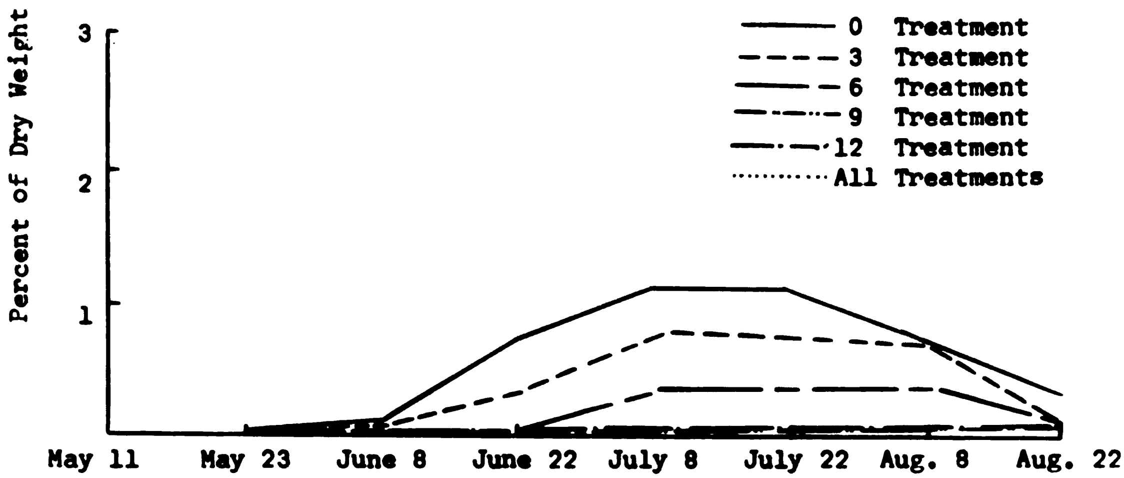
percent and 1.02 percent respectively. Nitrogen treatments did not differentially lower the carbohydrate reserves of common kentucky bluegrass and Pennlawn creeping red fescue. The glucose, fructose and sucrose fractions were affected little by nitrogen treatments. Temperature may have been the dominant factor. Oligosaccharide other than sucrose (Figure 5 and Appendix Table II), present only in Merion and Toronto creeping bentgrass leaves in appreciable concentrations, showed an inverse relationship between nitrogen treatments and oligosaccharide concentration. Under no treatment did oligosaccharide show much variation. More extreme fluctuations were caused by other environmental factors.

Total carbohydrate values for Pennlawn (Appendix Table X) contained a lag in peaks for the 0 and 3 treatments relative to 6, 9 and 12 treatments. Soil temperatures prior to June 8 were considerably below 70 degrees F. Soil organisms involved in organic decomposition and release of nitrogen may have responded to the warmer soil temperatures around June 8. Soluble nitrogen applied April 15, May 15 and May 31 in the 6, 9 and 12 treatments may have overcome this nitrogen shortage and stimulated plant enzyme systems of produce carbohydrate. The 0 and 3 treatments lacked sufficient soluble nitrogen to initiate this enzyme activity. Warmer temperatures June 8 to 22 appeared to accomplish an effect similar to the 6, 9 and 12 treatments June 8. All treatments steadily decreased in percent total carbohydrate as soil temperatures remained near 80 degrees F.

Results of the regrowth in the dark experiment lacked the precision and specificity of chemical determinations. The tendency of values between treatments to overlap decreased their reliability. Data relating

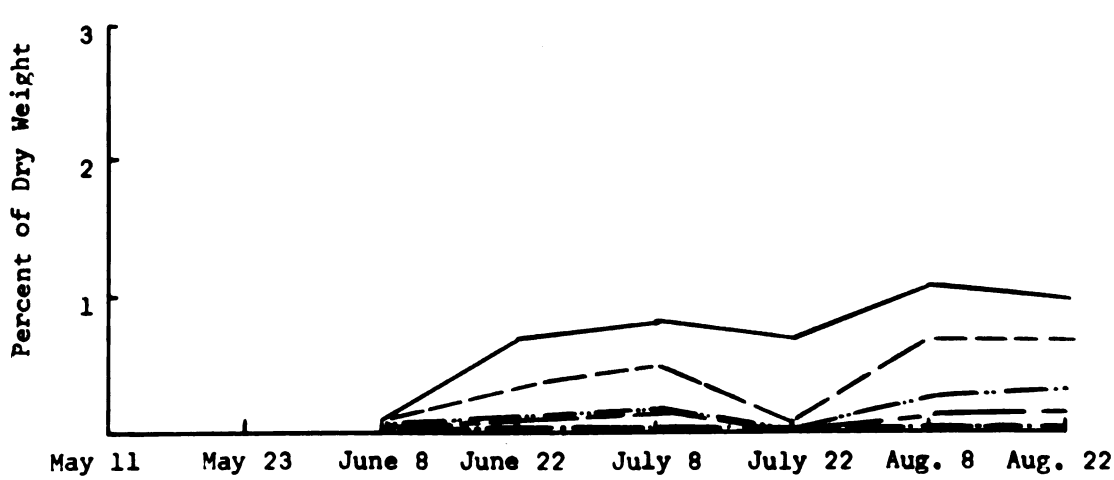
Figure 5. Oligosaccharide Minus Sucrose
Merion Kentucky Bluegrass

A



Toronto Creeping Bentgrass

B



treatments to height lacked consistency. However, the lower nitrogen treatments produced the most dry matter. This agrees positively with chemical indications that the lower nitrogen treatments generally contained the maximum reserve carbohydrates.

Total leaf carbohydrate concentrations for all four grasses with 80 degree temperatures and the 12 pound treatment only decreased to levels one-quarter or greater of their seasonal maximum. Total carbohydrates did not decline to zero. This suggests the possibility that carbohydrates are not the only factor which might limit growth under high temperature conditions. Meyer and Anderson's (19) exhaustive coverage of temperature-plant relationships exemplifies the complexity of the problem. A logical explanation could possibly be found by considering the relationships between temperature and the reaction rate of an enzyme-catalyzed reaction. Enzyme-catalyzed reaction rates are temperature dependent due to temperature affecting the kinetic energies of the reactants and the enzymes' protein structure. Temperatures in the 80 F. range and higher appeared to exert an adverse effect on carbohydrate reserves of the four cool season grasses studied, and this effect exceeded that attributable to the applied nitrogen treatments.

SUMMARY AND CONCLUSIONS

1. A technique for quantitative carbohydrate determinations using densitometer readings from paper chromatograms was shown to be practical for analyzing large numbers of samples.

2. The greenhouse environment produced leaf tissue considerably lower in oligosaccharide than were field samples.

3. The polysaccharide present in all grasses studied was identified to be a glucopolyfructan and quantitatively analyzed by a simple colorimetric test.

4. Effects attributable to nitrogen treatments were observed in the oligosaccharide and polysaccharide fractions containing more than two hexose moieties. Oligosaccharide concentrations greatly exceeded fructosan under field conditions. The di- and monosaccharides did not show differential concentrations related to nitrogen treatments.

5. The addition of more than one and one-half pounds of actual nitrogen per thousand square feet produced excess nitrogen conditions, as evidenced by parallel carbohydrate responses.

6. Nitrogen differentials for an experiment in time should be applied periodically to maintain relatively constant treatment differentials.

7. The technique of measuring plant food reserve as indicated by regrowth in the dark, produced results which, although less specific, agreed with chemically determined carbohydrate reserve values, particularly the oligosaccharide and polysaccharide concentrations.

8. Temperatures in the 80 F. range and higher appeared to exert an adverse effect on the carbohydrate reserves of the four cool season grasses studied, and this effect exceeded that attributable to the applied nitrogen treatments.

9. Under the conditions of this study and the nitrogen treatments used, the total carbohydrate level in the leaves of the four grasses did not appear to be present in concentrations which were inadequate for growth.

LITERATURE CITED

1. Archbold, H. K. 1940. Fructosans in monocotyledons. A review. New Phytol. 39:185-219.

2. Bacon, J. S. D. 1959. The trisaccharide fraction of some monocotyledons. Biochem. J. 73:507-514.

3. Brown, E. M. 1943. Seasonal variations in the growth and chemical composition of kentucky bluegrass. Missouri Exp. Stat. Bull. 360.

4. De Cugnac, A. 1931. Recherches sur les glucides des Graminees. Ann. Sci. Nat. 13:1-129.

5. Dubois, M., Cilles, K. A., Hamilton, J. K., Ribers, P. A. and Smith, Fred. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

6. Ehara, Kaoru and Tanaka, Shigeyuki. 1961. Effect of temperature on the growth behavior and chemical composition of warm- and cool- season grasses. Proc. Crop Sci. Soc. Japan. 29:304-306.

7. Giovannozzi-Sermanni, Giovanni. 1956. A new solvent for quantitative paper chromatography of sugars. Nature. 177:586-587.

8. Graber, L. F., Nelson, N. T., Leukel, W. A. and Albert, W. B. 1927. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wisc. Agr. Exp. Stat. Bull. 80.

9. Hansen, R. G., Forbes, R. M. and Carlson, Don M. 1958. A review of the carbohydrate constituents of roughages. NCR Pub. 88.

10. Harrison, C. M. 1934. Response of kentucky bluegrass to variations in temperature, light, cutting and fertilizing. Plant. Physiol. 9:83-106.

11. Jordan, E. E. 1959. The effect of environmental factors on the carbohydrate and nutrient levels of creeping bentgrass (Agrostis palustris). Purdue University. M. S. thesis (Unpub.).

12. Juska, F. J. and Hanson, A. A. 1961. Effects of interval and height of mowing on growth of Merion and common kentucky bluegrass (Poa pratensis L). Agron. J. 53:385-388.

13. Laidlaw, R. A. and Reid, S. G. 1952. Analytical studies on the carbohydrates of grasses and clovers. 1. Development of methods for the estimation of the free sugar and fructosan contents. J. Sci. Fd. Agric. 3:19-25.

14. Laidlaw, R. and Reid, S. G. 1950. Filter paper chromatography: Extraction of sugars from the paper at room temperature. Nature 166:476-477.

15. Lopatecki, L. E., Longair, E. L. and Kasting, R. 1962. Quantitative changes of soluble carbohydrates in stems of solid- and hollow-stemmed wheats. Can. J. Bot. 40:1223-1228.

16. Martin, A. J. P. and Synge, R. L. M. 1941. A new form of chromatogram employing two liquid phases. 1. A theory of chromatography. Biochem. J. 35:1358-1364.

17. McFarren, E. F., Brand, K., and Rutkowski, H. R. 1951. Quantitative determination of sugars on filter paper chromatograms by direct photometry. Anal. Chem. 23:1146-1149.

18. McRary, W. L. and Slattery, M. C. 1945. The colorimetric determination of fructosan in plant material. J. Biol. Chem. 157:161-167.

19. Meyer, B. S. and Anderson, D. B. 1952. Plant physiology. D. Van Nostrand Co., Inc. Princeton, New Jersey.

20. Paech, K. and Tracey, M. V. 1955. Modern methods of plant analysis. Zeiter Band vol. 2. Springer-Verlag.

21. Sprague, V. G. and Sullivan, J. T. 1950. Reserve carbohydrates in orchard grass clipped periodically. Plant Physiol. 25:92-102.

22. Sullivan, J. T. and Sprague, V. G. 1949. The effect of temperature on the growth and composition of the stubble and roots of perennial ryegrass. Plant Physiol. 24:706-718.

23. Troughton, A. 1957. The underground organs of herbage grasses. Bull. No. 44. Commonwealth Bureau Pastures and Field Crops, Hurley, Berkshire.

APPENDIX

Table I. Leaf oligosaccharide percentages determined on a dry weight basis for four grasses under five nitrogen treatments June 8 to August 22, 1963.

A Merion kentucky bluegrass

Treatment	June 8	June 22	July 8	July 22	Aug. 8	Aug. 22	Mean
0	3.86	6.37	6.28	5.63	4.54	4.43	2.60
3	3.47	5.56	5.66	5.04	4.48	4.51	2.39
6	.83	4.66	4.98	4.31	3.79	4.19	1.90
9	1.14	4.57	4.29	3.23	2.70	3.63	1.63
12	1.52	4.23	4.38	3.43	2.42	3.72	1.64
Mean	1.08	2.54	2.55	2.16	1.79	2.05	

B Toronto creeping bentgrass

Treatment							
0	2.79	1.86	4.72	4.23	4.42	6.21	2.02
3	2.25	1.11	4.24	2.97	3.68	4.81	1.59
6	1.93	.14	3.85	2.56	2.89	4.31	1.31
9	1.61	0	3.41	2.36	2.39	3.42	1.10
12	1.61	0	3.13	2.34	2.03	3.34	1.04
Mean	1.02	.31	1.94	1.45	1.54	2.21	

C Pennlawn creeping red fescue

Treatment							
0	1.88	4.36	4.92	3.50	2.76	4.52	3.66
3	1.68	3.56	3.42	4.08	2.64	4.08	3.28
6	1.78	3.28	3.22	3.52	2.94	3.28	3.00
9	2.20	3.22	3.16	3.42	3.40	3.20	3.10
12	3.34	2.86	2.94	3.50	2.92	3.50	3.18
Mean	2.22	3.46	3.53	3.60	3.13	3.72	

D Common kentucky bluegrass

Treatment							
0	3.70	3.78	4.22	3.34	3.30	4.30	3.77
3	1.94	4.78	4.46	3.54	3.84	4.42	3.83
6	3.04	4.84	4.40	3.28	3.46	3.78	3.80
9	4.30	5.46	4.24	3.22	3.88	3.96	4.18
12	2.78	5.46	4.36	3.30	4.16	4.34	3.92
Mean	3.15	4.86	4.34	3.34	3.73	4.16	

Table II. Leaf Oligosaccharide minus sucrose percentages determined on a dry weight basis for four grasses under five nitrogen treatments June 8 to August 22, 1963.

A Merion kentucky bluegrass

Treatment	June 8	June 22	July 8	July 22	Aug. 8	Aug. 22	Mean
0	.14	.75	1.12	1.12	.75	.29	.70
3	.08	.37	.75	.74	.71	.15	.47
6	0	.07	.37	.37	.38	.08	.21
9	0	0	0	.06	0	.07	.02
12	0	0	0	0	0	.06	.01
Mean	.04	.24	.45	.46	.37	.13	

B Toronto creeping bentgrass

Treatment							
0	.07	.66	.80	.67	1.06	1.0	.71
3	.07	.34	.46	.06	.67	.67	.38
6	0	..07	.13	0	.26	.34	.13
9	0	0	.11	0	.13	.14	.06
12	0	0	.05	0	.06	.14	.04
Mean	.03	.21	.31	.15	.44	.46	

C Pennlawn creeping red fescue

Treatment							
0	0	.69	.70	.01	0	.69	.35
3	0	.35	.40	.35	0	.21	.22
6	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
Mean	0	.21	.22	.07	0	.18	

D Common kentucky bluegrass

Treatment							
0	0	.07	.31	0	0	0	.06
3	0	.07	.31	0	0	0	.06
6	0	.07	.11	0	0	0	.03
9	0	.08	.08	0	0	0	.03
12	0	.08	.07	0	0	0	.03
Mean	0	.07	.18	0	0	0	

APPENDIX

Table III. Analysis of variance for oligosaccharide concentrations in the leaves of Toronto creeping bentgrass under five nitrogen treatments, June 8 to August 22, 1963

Source	DF	MS
Replication (R)	1	.0052
N treatments (N)	4	1.9352 **
RN, E_1	4	.0044
Dates (D)	5	4.6011 **
RD, E_2	5	.0546
ND	20	.0404**
RND, E_3	20	.0114

** Significant at the 1 percent level

* Significant at the 5 percent level

Table IV. Analysis of variance for oligosaccharide concentrations in the leaves of Merion kentucky bluegrass under five nitrogen treatments, June 8 to August 22, 1963

Source	DF	MS
Replication (R)	1	.1540
N treatments (N)	4	2.3451 **
RN, E_1	4	.0398
Dates (D)	5	3.0245 **
RD, E_2	5	.1766 *
ND	20	.1200
RND, E_3	20	.0571

** Significant at the 1 percent level

* Significant at the 5 percent level

Table V. Mean values for height (inches) and dry weight (grams) for regrowth in darkness of four grasses under five nitrogen treatments, June 15 to July 11, 1963.

Pounds nitrogen per thousand square feet	Toronto creeping bentgrass		Pennlawn creeping red fescue		Merion kentucky bluegrass		Common kentucky bluegrass	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
0	2.63	.28	.63	.04	3.25	.37	2.88	.20
3	1.63	.32	3.38	.04	3.75	.45	2.13	.12
6	1.25	.33	1.13	.04	5.00	.45	2.63	.16
9	.63	.15	1.13	.01	4.38	.30	2.38	.12
12	1.25	.28	1.5	.02	3.38	.21	2.00	.14

Table VI. A comparison of two methods for soluble carbohydrate extraction using a Soxhlet apparatus versus a Buchner-flask-funnel arrangement.

		Percent of Dry Weight			
	Method	Sucrose	Glucose	Fructose	Fructosan
Trial A *Poa pratensis*	Buchner Flask	1.5	-	.04	.115
	Soxhlet App.	1.41	-	.08	.035
Trial B *Poa pratensis*	Buchner	1.22	-	-	.04
	Soxhlet	1.08	-	-	.025

Table VII. Leaf carbohydrate percentages determined on a dry weight basis occurring in Merion kentucky bluegrass under three single application nitrogen treatments, August 5, 1963

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Oligosaccharide	Fructosan	Total Carbohydrate
Aug. 5	0	3.46	.30	-	4.21	.86	5.37
	6						
	12						
Aug. 6	0	3.02	.57	-	3.76	.84	5.17
	6	2.94	.28	-	3.69	.90	4.82
	12	2.43	.48	-	3.18	.84	4.50
Aug. 8	0	2.87	.45	-	3.61	.52	4.58
	6	2.28	.42	-	2.58	.59	3.59
	12	2.28	.28	-	2.58	.60	3.46
Aug. 10	0	2.21	.21	-	2.66	.66	3.53
	6	1.52	.30	-	1.52	.36	2.18
	12	2.43	.42	-	2.43	.28	3.13
Aug. 12	0	3.03	.63	-	3.03	.57	4.23
	6	2.79	.30	-	2.79	.10	3.19
	12	2.90	.36	-	2.90	.12	3.38
Aug. 16	0	2.46	-	-	3.58	.37	3.95
	6	2.30	-	-	2.30	.01	2.31
	12	2.30	-	-	2.30	.01	2.31

Table VIII. Leaf carbohydrate percentages determined on a dry weight basis occurring in common Kentucky bluegrass under three single application nitrogen treatments, August 5, 1963

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Oligosaccharide	Fructosan	Total Carbohydrate
Aug. 5	0	2.33	-	-	2.33	.06	2.39
	6						
	12						
Aug. 6	0	2.39	-	-	2.39	.03	2.42
	6	2.27	-	-	2.27	.03	2.30
	12	1.70	-	-	1.70	.03	1.73
Aug. 8	0	1.64	-	-	1.64	.02	1.66
	6	1.53	-	-	1.55	.02	1.50
	12	1.76	-	-	1.76	.01	1.77
Aug. 10	0	1.98			1.98	.02	2.00
	6	1.70			1.70	.01	1.71
	12	-	-	-	-	-	-
Aug. 12	0	1.7	-	-	1.7	.03	1.73
	6	1.55	-	-	1.55	.02	1.57
	12	1.94	-	-	1.94	.01	1.95
Aug. 15	0	1.79	-	-	1.79	.03	1.82
	6	1.79	-	-	1.79	.01	1.80
	12	2.15	-	-	2.15	.02	2.17

Table IX. Leaf carbohydrate percentages determined on a dry weight basis occurring in Toronto creeping bentgrass under three single application nitrogen treatments, August 5, 1963.

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Oligosaccharide	Fructosan	Total Carbohydrate
Aug. 5	0	2.37	.13	-	2.37	.07	2.57
	6						
	12						
Aug. 6	0	1.36	.04	-	2.36	.08	2.48
	6	1.37	-	-	2.32	.06	2.38
	12	.97	-	-	1.84	.03	1.87
Aug. 8	0	1.75	.08	-	2.05	.09	2.22
	6	.55	-	-	.93	.02	.95
	12	.99	-	-	1.25	.03	1.28
Aug. 10	0	1.20	-	-	2.20	.06	2.26
	6	1.05	-	-	1.05	.02	1.07
	12	1.17	-	-	1.17	.01	1.18
Aug. 12	0	1.12	-	-	2.10	.04	1.23
	6	.83	-	-	.83	0	.83
	12	.36	-	-	.96	0	.96
Aug. 16	0	1.44	-	.27	2.54	.07	2.88
	6	1.44	-	.08	1.44	.02	1.54
	12	1.45	-	.15	1.49	0	1.64

Table X. Leaf carbohydrate percentages determined on a dry weight basis occurring in Pennlawn creeping red fescue under three single application nitrogen treatments, August 5, 19[illegible]

Date	Pounds Nitrogen Per Thousand Square Feet	Sucrose	Glucose	Fructose	Oligosaccharide	Fructosan	Total Carbohydrate
Aug. 5	0	1.22	.64	-	1.22	.01	1.87
	6						
	12						
Aug. 6	0	1.10	.28	-	1.10	.02	1.40
	6	1.04	.22	-	1.04	0	1.26
	12	1.00	.31	-	1.00	0	1.31
Aug. 8	0	.83	.75	-	.83	0	1.58
	6	.92	.81	-	.92	.01	1.74
	12	.68	.63	-	.68	.01	1.32
Aug. 10	0	1.58	.31	-	1.58	0	1.89
	6	1.15	1.04	-	1.15	0	2.19
	12	.96	.97	-	.96	0	1.93
Aug. 12	0	.92	.78	-	.92	.02	1.72
	6	.81	.67	-	.81	.01	1.49
	12	1.13	1.06	-	1.13	0	2.19
Aug. 14	0	2.17	1.28	.15	2.17	0	3.60
	6	2.14	1.13	.19	2.24	0	3.57
	12	2.14	1.49	-	2.14	0	3.63