THE INFLUENCE OF TEMPERATURE, CULTURAL FACTORS, AND ANALYTICAL TECHNIQUES ON CARBOHYDRATE LEVELS IN TURFGRASSES

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ABSTRACT

THE INFLUENCE OF TEMPERATURE, CULTURAL FACTORS, AND ANALYTICAL TECHNIQUES ON CARBOHYDRATE LEVELS IN TURFGRASSES

By

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Total nonstructural carbohydrates were extracted from freeze-dried and heat-dried leaf tissue of Merion Kentucky bluegrass (Poa pratensis L.), Toronto creeping bentgrass (Agrostis palustris Huds.), and Tifgreen bermudagrass (Cynodon dactylon X C. transvaalensis) using 20 C water, 100 C water, and a modified Weinmann method. The anthrone, phenol-sulfuric, and Nelson's arsenomolybdate colorimetric procedures, and the Shaeffer-Somogyi copper-iodometric titration carbohydrate analysis techniques were compared. The three extraction techniques were comparable for Merion Kentucky bluegrass and Toronto creeping bentgrass; the modified Weinmann was more accurate than water for carbohydrate extraction of Tifgreen bermudagrass which had a high starch content. Phenol-sulfuric carbohydrate analysis resulted in higher carbohydrate levels, while the other three methods were comparable. | Carbohydrate losses were greater from heat-drying than from freeze-drying.

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Effects of cutting height, cutting frequency, delayed drying, and time of harvest in relation to the photoperiod were evaluated with Merion Kentucky bluegrass. Carbohydrate levels were slightly higher in Merion Kentucky bluegrass grown at a 5-cm cutting height compared to 2.5 cm when grown at 20 C, and higher at 2.5 cm when grown at 35 C. A weekly or semiweekly cutting frequency did not significantly alter carbohydrate levels. Delaying drying for 2 hours after sampling increased carbohydrate losses about equally at growth temperatures of 20 and 35 C. Carbohydrate levels in samples harvested from 0 to 14 hours after initiation of the light period increased over three fold when grown at 20 C. At a soil temperature of 35 C the carbohydrate level increased less than two fold.

Water-soluble carbohydrates (WSC) and dry matter production of Merion Kentucky bluegrass were evaluated at soil temperatures of 20, 25, 30, and 35 C. Further experiments at 20 and 35 C included sampling (a) at 2 and 10 hours after the beginning of the photoperiod; (b) individual leaf, stem, and root components; and (c) at photoperiods of 18, 16, 14, 12, and 10 hours. Photosynthetic and respiratory rates were measured at test temperatures of 20 to 40 C in Merion Kentucky bluegrass preconditioned at soil temperatures of 20 and 35 C. Maximum shoot growth occurred at 25 C and highest WSC levels at 35 C. Carbohydrate levels were higher at 35 C compared to 20 C in

several additional studies. Merion Kentucky bluegrass leaves sampled 2 hours after initiation of the light period had significantly higher WSC levels at 35 C than at 20 C, whereas samples collected at 10 hours had significantly higher WSC levels at 20 C than at 35 C. Thus, the time of sampling in relation to the photoperiod will alter the relative rate of WSC at an optimal and supraoptimal temperature. Merion Kentucky bluegrass roots contained less than half the WSC levels found in either the leaves or stems at both 20 and 35 C. WSC carbohydrates were higher at 35 C than at 20 C in all photoperiods except the longest (18 hours). Photosynthetic rates increased from 20 to 25 C and then decreased at higher test temperatures. The decrease was greater in grasses preconditioned at 20 C than those at 35 C. The rate of photosynthesis in Merion Kentucky bluegrass plants at 20 C was higher than at 35 C for all test temperatures except 40 C. Respiration increased as test temperatures increased from 20 to 40 C and was higher at all test temperatures in grasses preconditioned at 20 C.

In this investigation the carbohydrate levels in Merion Kentucky bluegrass remained high at supraoptimal temperatures, and the rate of photosynthesis exceeded respiration. Factors other than exhaustion of carbohydrate reserves caused growth reduction of Merion Kentucky bluegrass at supraoptimal temperatures.

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DEDICATION

To RUTH ANN and KARL DAVID

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INTRODUCTION

Carbohydrates are the primary source of reserve energy stored in grasses. These reserves accumulate during periods of high photosynthetic rates when carbohydrates are synthesized more rapidly than they are utilized in plant respiration and synthesis of structural carbohydrates and proteins. Conversely, these reserves are utilized by the plant during periods when carbohydrate utilization exceeds synthesis. The specific level of carbohydrate reserves may be influenced by numerous cultural and environmental parameters.

There has been a widely accepted hypothesis that supraoptimal temperatures (temperatures higher than the optimum for growth) have an adverse effect on carbohydrate reserves in grasses. The hypothesis assumes that carbohydrates accumulate at optimal temperatures because net photosynthesis exceeds net respiration, while net respiration and carbohydrate utilization exceeds the carbohydrates produced by photosynthesis at supraoptimal temperatures, causing a deficit. It has been postulated that carbohydrates are gradually depleted over a period of time and

that carbohydrate exhaustion is the cause of growth reduction and death of grasses at supraoptimal temperatures.

Recent data does not support this theory. This investigation was initiated to evaluate how the carbohydrate level is influenced by (a) carbohydrate extraction and analysis techniques, (b) several cultural factors, (c) sampling time and photoperiod, (d) optimal and supraoptimal temperatures, and (e) 20 and 35 C preconditioning temperatures on photosynthesis and respiration. The results obtained should assist in resolution of the apparent contradictions in the existing studies concerning heat stress physiology of grasses.

LITERATURE REVIEW

Carbohydrate Extraction and Analysis

Numerous plant carbohydrate extraction and analysis techniques have been reported in the literature to ascertain the quantity of carbohydrates in plant tissue. In certain cases the form of carbohydrate stored by a given plant dictates the appropriate extraction and analysis techniques. In others, it may depend on the convenience and judgment of the investigator. This has led to several techniques being used to investigate similar problems and has made it difficult to evaluate critically data from different laboratories.

In cultural studies it is generally desirable to evaluate the total nonstructural carbohydrate (TNC) content of the plant, since this is an estimate of the potential energy readily available to the plant for metabolic processes. A knowledge of the form of carbohydrate stored in the plant is needed to analyze this fraction. Grasses from tropical and subtropical origins, such as the tribes Eragrosteae and Chlorideae accumulate starch; while grasses

from a temperate origin, such as those in the tribes Festuceae, Hordeae, and Avenaeae accumulate fructosans (46). TNC from legume or grass species that accumulate starch has been extracted with acid solutions (20, 48) or diastatic enzyme preparations (20, 48, 58). TNC may be extracted from fructosan accumulators with dilute acid solutions or enzymes, but sugars and fructosans are also readily removed with water (20, 48). Fructosan-storing grasses may also accumulate very small amounts of water-insoluble starch (46).

Burris, Brown, and Blaser (9) compared the reserve carbohydrates in Midland bermudagrass (Cynodon dactylon L.) rhizomes using several enzymes and sulfuric acid concentrations. The enzyme extraction methods yielded from 4.3 to 5.7% total available carbohydrates, while the 0.2 N H_2SO_4 yielded approximately twice as much and the 2% H_2SO_4 approximately four times more than the enzyme. They utilized the analysis techniques of Nelson and Somogyi and the phenol-sulfuric acid method for carbohydrate analysis.

Schmidt and Blaser (44) extracted carbohydrates from Cohansey creeping bentgrass (Agrostis palustris Huds.) with 2% H_2SO_4. They obtained carbohydrate values of 39 to 53% for bentgrass stolons and 24 to 37% for leaf tissue. Extraction of monosaccharides by 80% ethanol yielded 2.6 to 5.1% in stolons and 1.9 to 4.1% in leaf tissue.

Carbohydrate levels in Merion, Fylking, and Newport Kentucky bluegrass (Poa pratensis L.) were evaluated by cold water extraction and anthrone analysis (61). Carbohydrate levels ranged from 14 to 22% for Merion, 14 to 18% for Fylking, and 12 to 16% for Newport. The variation was caused by a series of temperature regimes.

In a study with creeping bentgrass, monosaccharides were extracted using 85% ethanol and TNC with water (13). Anthrone reagent was utilized in both cases to determine the percent carbohydrate. Ethanol-soluble carbohydrates ranged from 5.2 to 8.1% and water-soluble carbohydrates from 6.1 to 9.8%, depending on the growth temperature.

Ten Kentucky bluegrass cultivars were evaluated for carbohydrate content by extraction with 0.1 N H_2SO_4 in a 100 C water bath and a quantitative determination made with the anthrone reagent (57). The 0.1 N H_2SO_4 extraction used was a modification of a more concentrated acid procedure previously used in that laboratory. Acid-soluble carbohydrate levels for the ten cultivars ranged from 10.0 to 24.2% at a day-night temperature of 23-15 C and from 7.0 to 14.1% after four weeks at 35-25 C.

Several of these methods would probably be acceptable for the determination of TNC. However, it would seem preferable to report results as acid-soluble carbohydrate, water-soluble carbohydrate, enzyme-soluble carbohydrate, etc., rather than as TNC. Several precautions should be

noted. A 0.2 N H_2SO_4 solution will destroy some of the fructose as well as hydrolyze some of the structural carbohydrate (20), resulting in an over-estimation of the actual TNC. In addition, water will not successfully extract starch from plant tissue and should not be used where starch is present in any significant quantity.

A number of research procedures and cultural practices have a profound influence on the carbohydrate content in grasses. It is of utmost importance, when making a comparative study, to standardize these variables. Several of these factors will be reviewed in the following paragraphs.

Drying Plant Samples

The method of drying plant samples may have a significant effect on the retention or loss of plant constituents. While heat-drying is more convenient, a lower percentage of TNC was retained compared to freeze-drying (42). Freeze-dried alfalfa (Medicago sativa L.) contained 10% TNC, alfalfa heat-dried at 100 C for 90 minutes and completed at 70 C contained 8% TNC, alfalfa dried at a constant 70 C temperature contained 7.4% TNC, and alfalfa dried at 27 C contained only 5.2% TNC. The procedure of heat-drying at 100 C for 90 minutes followed by complete drying at 70 C has been recommended for TNC analysis (42). Low temperatures must be maintained during the freeze-drying of plant tissue, and the plant sample must be sealed

during storage to keep the moisture content minimal, otherwise, interconversions of carbohydrates may occur.

Cutting Frequency and Height

Cutting frequency also has an effect on the carbohydrate content of the plant. Evers and Holt (16) found a slight decrease in carbohydrates when comparing a 5-week to a 3-week cutting frequency in kleingrass (*Panicum coloratum* L.). They also reported slightly higher carbohydrate levels at a 15-cm cutting height compared to 5 cm. Decreased shoot growth and loss of roots and rhizomes may result from frequent, severe defoliation of Kentucky bluegrass (21). Carbohydrates in bermudagrass and buffalograss (*Buchloe dactyloides* Nutt.) roots and rhizomes were also reduced by cutting the shoots (29). The same response has been found for orchardgrass (*Dactylis glomerata* L.) (49). Frequent defoliation of forage crops is commonly accepted to be detrimental to the vigor and survival of the plant.

Soil Moisture

It is desirable to keep environmental factors uniform when evaluating carbohydrate levels. Soil moisture or the internal plant water deficit may vary from one temperature to the next. Moisture stress may cause an increase in the carbohydrate level and a decrease in shoot growth. Soil moisture levels held at 10 to 30, 40 to 60,

and 80 to 100% of available water, showed a 50% reduction in shoot growth and a 3 to 8% increase in carbohydrates in orchardgrass at the low moisture level (8). This was attributed to a reduced shoot growth rate and energy demand. Analyses of roots and rhizomes showed that, in some cases, drought caused carbohydrate levels to increase about two fold (29). This data illustrates the importance of maintaining uniform soil moisture levels within an experiment when investigating carbohydrate levels.

Nitrogen

Nitrogen is an essential element for the growth of grasses. It increases dry matter production when added to the sward. However, the reduction in growth of Italian ryegrass (Lolium multiflorum Lam.) caused by high temperatures was even greater at high nitrogen levels (51). The nitrogen level also affects the quantity of soluble carbohydrates present. Orchardgrass and ryegrass had higher carbohydrate levels at low soil nitrogen levels and both decreased with added nitrogen (2, 49). Nitrogen fertilization reduced the fructosan concentration in tall fescue (Festuca arundinacea Schreb.) from 7.3 to 0.4% for the first harvest, while pentosans were reduced from 12.6 to 7.3% (32).

Several nutritional studies on Kentucky bluegrass have demonstrated an increase in growth but a decrease in foliar carbohydrate content with the addition of nitrogen

fertilizer (40, 56). Nitrogen fertilization reduced the levels of acid-extractable carbohydrates found in Cohansey creeping bentgrass (44). Low light intensities caused the same response (2, 44). Green and Beard (19) investigated the relationship between nitrogen nutrition and reserve carbohydrates in the leaves of both creeping bentgrass and Kentucky bluegrass. They found that oligosaccharides and fructosans decreased with added nitrogen while simple sugars were not significantly affected. Total carbohydrate content was also higher in most instances at reduced nitrogen levels. The literature demonstrates the necessity for maintaining uniform nitrogen levels in any experiments involving a carbohydrate assay.

Sampling Time

Krotkov (30) reported a maximum sugar content in wheat (Triticum aestivum L.) leaves between 3 and 6 p.m. The sucrose concentration was primarily responsible for the daily variation. He also reported greater diurnal variation in carbohydrate levels in young leaves compared to older leaves because of a decreased reducing sugar content in the latter.

Similar trends have been observed in forage grasses. Sucrose increased in ryegrass from about 5% at 9 a.m. to 7% at 3 and 6 p.m., while the fructosan content varied irregularly (55). Seventy percent of the daily increase in sucrose was lost between midnight and 3 a.m. The sugar and

starch content of alfalfa increased from morning to afternoon, but the variation in total sugar content was only about 1% (36). The diurnal fluctuation of carbohydrates in tall fescue has also been investigated. Water-soluble carbohydrates increased from 6% at 6 a.m. to 9% at 6 p.m. (26). In another study, the average sugar (mostly sucrose) level increased from about 8% at 6 a.m. to about 10% 12 hours later. The fructosan concentration did not vary diurnally. Almost 37% of the daily increase in sucrose was respired or translocated between 6 p.m. and midnight, and the remaining 63% after midnight (32). Water-soluble carbohydrates in Kentucky bluegrass increased from approximately 5% at 6 a.m. to 7% at 12 N and to 8% at 6 p.m. (26).

Only one investigation has been brought to the author's attention in which this phenomena was investigated under a turfgrass cultural system. During a 16-hour photoperiod and 15.6 C temperature, the fructose level in creeping bentgrass increased from 3.8 to 5.6%. Fructoses increased from 1.6 to 5.4% when the creeping bentgrass was grown at 26.7 C (28).

Significant carbohydrate changes occur in a relatively short period of time. Thus, another factor influencing the carbohydrate level of plant tissue is the time of sampling with respect to the photoperiod. An early morning sampling may differ from an afternoon sampling even if all other factors are kept constant. Samplings must

therefore be made at a comparable time relative to initiation of the photoperiod in order to have valid comparisons.

Effects of Temperature on Growth

The effects of temperature on various growth parameters have been studied extensively. Sullivan and Sprague (53) grew perennial ryegrass (Lolium perenne L.) at day-night temperature regimes of 15.6-10, 21.1-15.6, 26.7-21.1, and 32.2-26.7 C. Their data were obtained 21, 28, and 40 days after an initial clipping. Root dry-matter production was greatest at the 15.6-10 C (day-night) temperature and lowest at the highest temperature regime. In the stubble component, the highest dry-matter production was at the lowest temperature, but lowest production was at 26.7-21.1 C (day-night). Dry-matter production of shoots was highest at day-night temperatures of 21.1-15.6 C and lowest at 32.2-26.7 C. Generally, dry-matter production of all three components decreased with increasing temperature.

The effect of temperature and nitrogen on growth of Kentucky bluegrass was examined at 15.6, 26.7, and 36.7 C (21). Cultures grown at 15.6 C with added nitrogen had similar dry weight production for five consecutive cuttings made at 10-day intervals. The cultures with added nitrogen grown at 26.7 C decreased in shoot growth with successive cuttings. Shoot growth was higher at 26.7 C than at 15.6 C only for the first 10-day sampling. The 36.7 C culture produced no growth following the first 10-day cutting. The

dry weight of the rhizomes at the end of the experiment decreased with increasing temperature while root weights ranked 15.6>36.7>26.7 C.

Three Kentucky bluegrass cultivars, Merion, Fylking, and Newport, were grown at day-night temperature regimes of 27-21, 27-16, 18-12, and 16-7 C. Shoot growth was greatest at the warmer temperature and decreased with decreasing temperature, except that in Fylking and Newport the shoot growth was slightly higher at day-night temperatures of 27-16 C than at 27-21 C. Dry matter produced by Merion was significantly greater than for the other two cultivars (61).

Growth of creeping bentgrass also varied with temperature treatments. Leaf growth was greatest at 21.1 C, less at 15.6 C, and considerably reduced at 26.7 C (28). Growth rate per day decreased with time at all three temperatures under the conditions of the investigation.

McKell, Youngner, Nudge, and Chatterton (35) grew Coastal bermudagrass and Newport Kentucky bluegrass at day-night temperature regimes of 13-7, 18-13, 30-18, and 30-24 C. Shoot growth of bermudagrass increased as temperature increased. Shoot growth of Newport Kentucky bluegrass was greatest at 18-13 C (day-night) and increased with each sampling date. Shoot growth at the other three temperatures was similar on the first sampling date and only varied slightly on subsequent sampling dates.

The most shoot growth in Cohansey creeping bentgrass occurred after 45 days at 36 C, followed by 24 and 12 C. The highest root production was at 12 C, followed by 24 and 36 C. Root and shoot growth of bentgrass increased as temperatures were decreased from 36 C. However, when cultures maintained at 12 C were grown at higher temperatures, shoot growth increased while root growth decreased (44).

Stuckey (52) observed accelerated maturation of colonial bentgrass (Agrostis tenuis Sibth.) roots caused by high soil temperature. She stated that early maturation and death of the root system was apparently responsible for plant death at high temperature.

Maximum shoot production of Kentucky bluegrass was observed at an average soil temperature of 15.6 to 17.8 C. Very little shoot growth occurred at soil temperatures less than 10 C (6).

Watschke, Schmidt, and Blaser (56) observed growth of Pennstar, Kenblue, Nugget, 110, and 124 Kentucky bluegrass cultivars at day-night temperature regimes of 18-10, 27-18, and 35-20 C. Shoot growth was least at the highest temperature and highest at the two lower temperatures. Maximum root production occurred at a day-night temperature of 27-18 C and least at 35-20 C. They also observed enhanced tolerance to heat stress when the grasses were preconditioned at cool temperatures. Kentucky bluegrass

cultivars selected from a warm climate were better adapted to heat stress.

Ten Kentucky bluegrass cultivars were grown at a 23-15 C day-night temperature, then changed to 35-25 C. The shoot growth of all cultivars was higher at 35-25 C the first week, but less on succeeding weekly harvests (57).

Duff (13) measured the weekly shoot growth of Toronto creeping bentgrass at five day-night temperature regimes: 20-10, 25-15, 30-20, 35-25, and 40-30 C. Shoot growth decreased with each increment of increased temperature, but the weight/unit leaf area ratio increased. Leaf width and leaf length both decreased with increasing temperature.

Beard and Daniel (3) reported the root growth of C-52 creeping bentgrass to be similar at 15.6, 21.1, and 26.7 C but reduced at 32.2 C. However, total root production decreased as temperature increased.

Temperature also affects the type of leaf produced. Ryegrass leaves grown at a day-night temperature of 21.1-15.6 C were vigorous with a good color. The leaves were shorter at 15.6-10 C (day-night) while at 26.7-21.1 C they were spindly and lacked vigor, and at 32.2-26.7 C they were still shorter and a very dark green color (53).

Profuse, long, succulent Kentucky bluegrass leaves were produced at a 15 C soil temperature. However, plants had short, rigid, erect leaves when grown at a 35 C soil

temperature along with limited leaf production and bud initiation (10).

Toronto creeping bentgrass grown at 30-20 C (day-night) was vigorous, dense, and had good color. The leaves were more upright at 35-25 C (day-night) and had a bristle-like appearance and a decreased density. Leaves at 40-30 C (day-night) were similar with a further decrease in density and a darker green color (13).

Fluctuations in temperature have profound effects on plant growth, particularly when the temperature exceeds the optimum range (termed supraoptimum) for a given species.

Effects of Temperature on Carbohydrate Levels

The carbohydrate content of plants has been widely investigated in relation to many cultural and environmental variables. In general, factors which promote leaf growth cause a reduction in carbohydrate levels or cause levels to remain at previously existing low levels (1, 7, 8, 28).

Seasonal fluctuations in temperature may significantly affect the carbohydrate level in grasses. Brown (6) reported greater carbohydrate production in Kentucky bluegrass during the cool weather of spring, resulting in a surplus. A net loss of carbohydrates from roots and rhizomes occurred during the summer, with storage occurring again in the fall.

The fructosan content in orchardgrass grown at 30 C was 7% in the blades and 13.3% in the sheaths (14). When grown at 5 C, the fructosan content was 0.7% and 1.7%, respectively.

The highest percentage of carbohydrates in ryegrass was found in the stubble, with lesser amounts in the leaves and roots. Maximum carbohydrate accumulation occurred in ryegrass at a day-night temperature of 2.1-15.6 C, with a rapid decrease in carbohydrates at temperatures above the optimum for growth (53). Harrison (21) also attributed reduced growth at higher temperatures to the exhaustion of carbohydrates.

Summer growth of white clover (Trifolium repens L.) was reduced by a decline in branching. Although the carbohydrate supply also decreased, this did not appear to be the limiting factor in summer branching (4).

Zanoni, Michelson, Colby, and Drake (62) showed the seasonal fluctuation in carbohydrate levels to be directly related to soil temperature. Merion Kentucky bluegrass contained an increasing carbohydrate level from late spring to mid-summer, a sharp drop in late summer, and an increase in the fall. Penncross creeping bentgrass, Kingstown velvet bentgrass, and Astoria colonial bentgrass contained a depressed carbohydrate level during spring and summer, and an increase in the fall.

The total fructose content in creeping bentgrass at three temperatures was in the order of 15.6>26.7>21.1 C (28). Green (18) reported an adverse effect on carbohydrate levels in cool season grasses grown at 26.7 C. The levels were apparently not sufficiently low to inhibit growth.

Carbohydrate levels in five Kentucky bluegrass cultivars decreased as the growth temperature was increased from day-night regimes of 18-10 to 27-18 to 35-20 C (56). The authors also reported that cultivars with a high carbohydrate content best supported growth at the higher temperature.

The weekly foliar carbohydrate level in Kentucky bluegrass decreased as the day-night growing temperature was increased from 23-15 to 35-25 C (57). Although the carbohydrate level for ten cultivars ranged from 7% to 14.1% after 4 weeks at the higher temperature, the authors still attributed reduced dry matter production to a carbohydrate content approaching threshold levels. Cultivars exhibiting a more gradual decline in carbohydrate content also had a slower decline in growth.

Although Youngner and Nudge (61) reported that temperatures favorable to growth resulted in lower carbohydrate reserves in Kentucky bluegrass, they did not include supraoptimal temperatures for comparative purposes. They report highest shoot growth in Merion Kentucky

bluegrass at day-night temperatures of 27-21 and 27-16 C, and highest carbohydrate levels at day-night temperatures of 16-7 and 18-13 C. McKell et al. (35) also measured lower carbohydrate levels at higher temperatures.

The acid-extractable carbohydrates in Cohansey creeping bentgrass stolons decreased from 43.4% at 12 C to 31.8% at 36 C. The carbohydrate level in leaves decreased as temperature increased while the monosaccharide content in stolons and leaves was in the order 24>36>12 (44).

Brown and Blaser (8) found carbohydrates in orchardgrass to be no lower at 35 C than at 24 C. They attributed this to more severe moisture stress at 35 C which may have counteracted the effects of the higher temperature.

In the only data available showing the carbohydrate content to be greater in plants grown at higher temperatures, Duff (13) reported that the 85% ethanol-soluble carbohydrate level in creeping bentgrass increased from 5.2 to 8.1%, and water-soluble carbohydrates increased from 6.1 to 9.8% at day-night temperature regimes of 20-10 C and 40-30 C, respectively. Stoin (51) also concluded that the carbohydrate content was not the causal factor in reduced growth at supraoptimal temperatures.

Photosynthesis and Respiration

The rate of photosynthesis is affected not only by temperature, but by light intensity and quality, carbon

dioxide concentration, water availability, leaf age, and chlorophyll content. The degree to which a given temperature influences photosynthesis depends largely on the intensity of light and CO_2 availability. Above some optimum temperature, net photosynthesis will be depressed. El-Sharkay and Hesketh (15) found that between 30 and 45 C rapid net photosynthesis can occur for at least a short time.

Likewise, respiration is affected not only by temperature but also by available oxygen, an adequate energy source, plant injury or pests, light, and age of the plant. Respiration increases up to a certain point with increasing temperature. Beyond a certain temperature, which varies with the plant condition and species, respiration will decrease. In addition to respirational losses of carbohydrates, Hewitt and Curtis (23) have reported translocational losses.

Although photosynthesis and respiration rates both increase to a certain maximum temperature and then decrease, it has been widely accepted that the maximum rate for photosynthesis is at a lower temperature than for respiration. Thus, apparent photosynthesis equals zero at some point as temperature increases.

Maximum photosynthesis can occur in some green plants at a very high temperature. The photosynthetic rate in Tidestromia oblongifolia continued to increase up

to 47 C. A 3 C decrease in temperature caused a marked decrease in the photosynthetic rate at high light intensities. A very high rate of carbon dioxide fixation, 58 mg CO_2 $dm^{-2}hr^{-1}$ as an average of the whole plant, was measured (5).

Moss, Musgrave, and Lemon (38) showed respiration increased in corn (Zea mays L.) at a much greater percentage rate than photosynthesis as temperature increased up to 43.9 C. However, the magnitude of photosynthesis was so much greater that net assimilation was greater at the higher temperature. Hew, Krotkov, and Canvin (22) reported little effect of temperature on the rate of apparent photosynthesis in corn.

A wide difference in photosynthesis between temperate and tropical origin forage grasses was reported by Murata and Iyama (39). Apparent photosynthesis in Italian and perennial ryegrass was highest at about 5-15 C, and at 35 C for bermudagrass and bahiagrass (Paspalum notatum Flugge). Respiration of all species increased with increasing temperature through 40 C.

The maximum apparent photosynthetic rate for Seaside creeping bentgrass and common bermudagrass occurred at 25 C and 35 to 40 C, respectively. The relative rate for bentgrass was 64.6% at 15 C, a maximum 100% at 25 C, and still 62.2% at 40 C. The relative rates for bermudagrass were 54.9% at 15 C, 100% at 35 C, and 97.7% at 40 C (37).

In Cohansey creeping bentgrass grown at 32 klux, 80% more CO_2 was fixed at 24 C as compared to 12 C or 36 C (44).

Watschke, Schmidt, Carson, and Blaser (57) reported photosynthesis and respiration data for ten Kentucky bluegrass cultivars. They measured a photosynthetic rate of 12.3, a photorespiration rate of 7.5, and a dark respiration rate of 5.2 mg CO_2 $hr^{-1}g^{-1}$ dry leaf for Merion Kentucky bluegrass grown at a 35-25 C day-night temperature.

Duff (13) grew Toronto creeping bentgrass at day-night temperatures of 20-10 and 40-30 C, and measured photosynthesis and respiration rates manometrically at test temperatures of 20, 30 and 40 C. Both rates increased with increasing test temperature. The rate was higher in grasses from the higher growth temperature and the difference widened with increasing test temperature.

Woledge and Jewiss (59) studied the effect of growth temperature on subsequent photosynthesis in tall fescue. They observed that the optimum temperature for apparent photosynthesis was higher in leaves from plants grown at a high temperature regime than from a low one, and that at a series of test temperatures the leaves from the high growth temperature had a lower respiration rate. Their high day-night temperature regime was only 20-14 C and, thus, does not indicate whether there is a favorable photosynthetic or respiratory adaptive mechanism at supra-optimal temperatures.

An increasing chlorophyll content was reported for grasses grown at a series of day-night temperatures from 20-10 C to 35-25 C (13). The chlorophyll content decreased at 40-30 C, although it was still higher than at 20-10 C. Dry matter production and chlorophyll content did not correlate.

MATERIALS AND METHODS

Carbohydrate Extraction and Analysis Technique Study

Leaf samples of Merion Kentucky bluegrass, Toronto creeping bentgrass, and Tifgreen bermudagrass were collected in May 1971. The Kentucky bluegrass and creeping bentgrass samples were obtained from field plots at East Lansing, Michigan. Bermudagrass sod was obtained from Florida and grown in the greenhouse at East Lansing. The leaf samples collected were sufficiently large so that subsamples for all extraction and analysis technique comparisons came from one sample. Dry ice was added to one-half of the sample used for freeze-drying, while the other half was heat-dried at 100 C for 90 minutes and to dryness at 70 C. This heat-drying procedure proved effective previously (42). The dried samples were ground in a Wiley Mill having a 60 mesh sieve.

Carbohydrate Extraction Comparisons

Carbohydrates from Merion Kentucky bluegrass, Toronto creeping bentgrass, and Tifgreen bermudagrass

leaves were extracted using (a) 20 C water, (b) 100 C water, and (c) an enzyme. For the first method a 1-g sample was weighed, placed in a 250-ml flask, and 50 ml of 20 C water added for each of two replications. The flasks were stoppered and shaken for 1 hour on a Burrel Wrist-Action Shaker. The solution was suction-filtered through Whatman #42 filter paper in a Buchner funnel plus several rinses. Celite Analytical Filter-Aid was also layered on the filter paper to aid in the filtering process. The filtrate was poured into a volumetric flask and appropriate dilutions made so that 1 ml of the extract contained between 10 and 60 µg of carbohydrates. The 100 C extraction procedure was similar except that the flasks were placed in a boiling water bath for 1 hour.

The enzyme extraction procedure involved incubation at about 38 C for 44 hours with a 0.5% takadiastase enzyme solution. The method was first suggested by Weinmann (58). A modified procedure outlined by Smith (47) was used in this investigation.

Carbohydrate Analysis Comparisons

Four carbohydrate analysis techniques, (a) anthrone, (b) phenol-sulfuric, (c) Nelson's arsenomolybdate, and (d) Shaeffer-Somogyi, were utilized in conjunction with the three extraction techniques and three species used in this study. The first three analysis techniques were used on

extractions using both 20 and 100 C water, while the fourth was used in conjunction with the modified Weinmann extraction procedure.

The anthrone analysis technique proved to be a simple, rapid, and accurate method for the determination of total carbohydrates. Thus, it was chosen for use in subsequent experiments. The reaction of sulfuric acid, carbohydrates, and anthrone reagent to produce a green color was first used by Dreywood (11). The procedure described below is similar to that used by Trevelyan and Harrison (54) and Yemm and Willis (60). The anthrone reagent was prepared by dissolving 0.2 g of anthrone in 100 ml of a mixture prepared by adding 500 ml of concentrated sulfuric acid to 200 ml of distilled water. The reagent was shaken periodically for a 30 to 40 minute period and used within 1 hour. Five ml of the reagent was placed in a test tube using a 5-ml tilting dispenser. The tubes with reagent were chilled in an ice bath. One ml of the carbohydrate extract containing 10 to 60 µg of sugar was pipetted into each tube by carefully layering it on top of the reagent. After chilling, the tubes were inverted four times for complete mixing and chilled again. Consistent color development was achieved by covering each tube with a marble, placing in a boiling water bath for exactly 10 minutes, and chilling again in the ice water. Absorbance was read

against a water blank in either a Beckman DU or a Hitachi Perkin-Elmer 139 UV-VIS Spectrophotometer at 620 nm. The unknown samples were prepared in triplicate. Carbohydrate content was computed from a standard curve obtained by measuring standard samples of 10, 20, 30, 40, 50, and 60 µg of fructose. These known samples were also analyzed each time to insure correlation with the standard curve.

The procedures for Nelson's arsenomolybdate and the phenol-sulfuric acid method (12) are outlined by Hodge and Hofreiter (25). The former is a colorimetric modification of a method by Somogyi while the latter was developed at the University of Minnesota.

The carbohydrate content of the enzyme extracts was determined by the Shaeffer-Somogyi copperiodometric titration method as outlined by Smith (47).

Factors Affecting Carbohydrate Levels

Mature sods of Merion Kentucky bluegrass were obtained from the field plots at East Lansing, Michigan, during the spring of 1972. Boxes 35 x 50 x 9 cm were lined with plastic having several holes punched in the center. About 2.5 cm of a modified sandy loam was placed in the box and the sod transplanted on top. This procedure provided greater moisture retention by the sod, and was particularly important for treatments involving supra-optimal temperatures. The sod was irrigated one or two times a day depending on the temperature. A complete

Hoagland's nutrient solution (24) was applied weekly. The sod was placed in a controlled environment chamber having a 14-hour photoperiod with the light intensity maintained at 30 klux. Two replications of leaf samples cut at 5 cm were obtained 1 hour after initiation of the light period. Carbohydrates were extracted using 20 C water and analyzed by the anthrone method.

Cutting Height

Leaf samplings at cutting heights of 5 and 2.5 cm were made for five consecutive weeks on Merion Kentucky bluegrass grown at 20 and 35 C. The sod had been preconditioned to these cutting schedules for 4 weeks prior to initiation of the study. Dry matter production and water-soluble carbohydrates were determined after freeze-drying.

Cutting Frequency

Leaf samples were obtained for this study on a weekly and semiweekly cutting schedule for a 5-week period. Dry matter production and water-soluble carbohydrates were determined following freeze-drying.

Delayed Drying

A uniform sample of leaf tissue was obtained from Merion Kentucky bluegrass sod grown at both 20 and 35 C. One-half of each sample was freeze-dried and the other portion heat-dried at 100 C for 1 hour and to dryness at

70 C. However, the drying process was delayed on subsamples for 15, 30, 60, and 120 minutes from the time of cutting. Water-soluble carbohydrate determinations were made and compared to subsamples in which drying was initiated immediately.

Diurnal Fluctuation

Both the water-soluble and 85% ethanol-soluble carbohydrate levels were monitored throughout a 14-hour photoperiod at 20 C. Two replications of leaf samples were obtained at 0-, 1-, 2-, 5-, 8-, 11-, and 14-hour intervals during the day and at 1, 3, and 10 hours during the dark period.

Effects of Temperature on the Carbohydrate Levels

The effects of optimal and supraoptimal temperatures on water-soluble carbohydrates were investigated through a series of studies as outlined below. Mature Merion Kentucky bluegrass sod was transplanted as outlined previously. The turf was irrigated at least daily and frequently twice daily at the higher temperature treatments to prevent soil water stress. Hoagland's nutrient solution (24) was applied weekly. The temperature treatments reported were soil temperatures, and were regulated in a controlled environment chamber having a light intensity of about 30 klux. All experiments were conducted at

a 14-10 hour day-night regime, except for the photoperiod and diurnal temperature studies. Two replications of leaf samples at a 5-cm cutting height were collected weekly from 1 to 2 hours after initiation of the light period. The tissue was freeze-dried, weighed for total dry matter, ground in a Wiley Mill to pass a 40 mesh screen, and water-soluble carbohydrates determined quantitatively by the anthrone procedure.

Carbohydrate Levels at Four Temperatures

Carbohydrates were monitored in Merion Kentucky bluegrass grown at 20, 25, 30, and 35 C. Weekly leaf samplings were made at each temperature regime for two consecutive weeks. The soil temperature was raised 5 C at the end of the second week at each temperature treatment. The same controlled environment chamber was utilized for the entire study. The light intensity was maintained at 26 klux.

Carbohydrate Levels at 20 vs. 35 C

An optimum shoot growth temperature for Merion Kentucky bluegrass and a supraoptimal temperature that caused a severe reduction in growth were chosen for further study. One controlled environment chamber was adjusted to produce a constant soil temperature of 20 C and a second chamber was set at a constant soil temperature of 35 C.

All other conditions in the chamber were similar including a 30 klux light intensity and a 14-hour photoperiod. The study was repeated using a new set of sod plugs. The temperature was adjusted to 20 C in the 35 C chamber after 8 weeks. The microenvironment temperature was checked with a Leeds & Northrup portable potentiometer and the leaf temperature with a Stoll-Hardy HL4 radiometer.

Diurnal Temperature Study

A controlled environment chamber was programmed for a 23-15 C day-night temperature and a 16-hour photoperiod. The temperature was raised to 35-25 C (day-night) after 3 weeks. Carbohydrate analyses were performed on weekly leaf harvests at both temperature regimes. These data were obtained for comparative purposes with data from other laboratories.

Morning vs. Afternoon Sampling Comparisons

Leaf harvests from bluegrass sods grown in 20 C chambers at a 14-hour photoperiod were made at 2 and 10 hours after initiation of the light period. The temperature in one chamber was changed to 35 C after 4 weeks while the other chamber was continued at 20 C. Quantitative carbohydrate determinations were then made on leaf tissue grown at 20 and 35 C and harvested both 2 and 10 hours into the light period.

In an additional study, turfs were preconditioned for 4 weeks at 20 and 35 C, respectively, and leaf harvests made at 3-hour intervals throughout a 14-hour day. The increase in carbohydrate levels during the day was compared at the two soil temperature treatments.

Carbohydrate Content in Leaves, Stems, and Roots

Merion Kentucky bluegrass was seeded in 11-cm diameter cups containing silica sand. After establishment in the greenhouse for 3 months, the cups were transferred to 20 and 35 C controlled environment chambers. The cups were placed in a pan containing water so that the water level was in contact with the lower surface of the sand. The turfs were irrigated twice daily. Three replications were harvested weekly and the leaves, stems, and roots separated. A stream of water and screens were used to separate the sand from the roots. The effect of temperature on the carbohydrate content of the leaves, stems, and roots was determined over a 5-week period.

Photoperiod Study

Ten controlled environment chambers were programmed at two temperature levels and five photoperiods. Five chambers were set for a soil temperature of 20 C and five at 35 C. One chamber at each temperature was adjusted for each of five photoperiods: 10, 12, 14, 16, and 18 hours. Two 36- x 50-cm boxes containing rooted,

mature sods were placed in each of the 10 chambers. Weekly harvests of two replications per treatment were made 2 hours after initiation of the light period on five consecutive weeks.

Photosynthetic and Respiratory Rate Study

Single plants seeded in the leaf, stem, and root study were transplanted to 6 oz. styrofoam cups containing silica sand. They were allowed to grow in the greenhouse until several tillers developed. The small cups were inserted in 10 oz. waxed cups containing water for better moisture control and placed in both 20 and 35 C controlled environment chambers.

An analytical system was arranged to measure the carbon dioxide content of a continuous air stream. An inlet hose was connected to a tank of compressed air and an outlet hose to a Beckman Model IR 214A CO_2 gas analyzer. The air flow was adjusted to 500 cc min^{-1} at the analyzer outlet. The CO_2 analyzer was connected to a Beckman 10" linear recorder. The analytical system was adjusted so that nitrogen gas registered zero on the recorder, while the carbon dioxide content of the compressed air caused a response of 50 units. The system also included a sealed, clear, plastic assimilation chamber.

The assimilation chamber was placed in a controlled environment chamber to facilitate temperature

regulation at 20, 25, 30, 35, and 40 C inside the assimilation chamber. The light intensity inside the assimilation chamber was 27 klux.

Following 1 and 4 weeks of conditioning at 20 and 35 C, two cups of grass were placed into the assimilation chamber. With the lights on, photosynthesis decreased the CO_2 content of the egress air. When the analyzing system reached a steady state, the lights were turned off to measure dark respiration. After equilibrium was reached for respiration, another set of replicates was analyzed. The shoots were removed from a set of replicates and the remainder analyzed to correct for soil and root effects.

The plants were harvested and the leaf area determined. They were also dried for dry-weight determinations. Knowing the area and weight, the CO_2 content of the compressed air, and the flow rate, photosynthesis or respiration could be determined as μg of CO_2 min^{-1} unit $area^{-1}$ and/or unit wt^{-1}.

RESULTS

Carbohydrate Extraction and Analysis Technique Study

Total nonstructural carbohydrates (TNC) in freeze-dried and heat-dried leaves of Merion Kentucky bluegrass were extracted using three methods and quantitatively analyzed by four procedures. The data in Table 1 indicates that the 20 C water extraction procedure on freeze-dried tissue generally resulted in slightly higher carbohydrate values than the 100 C water extraction. The enzyme extraction method (Modified Weinmann) resulted in values similar to both water extractions, except when analyzed by the phenol-sulfuric method. Carbohydrate analyses by anthrone, Nelson's arsenomolybdate, and Shaeffer-Somogyi methods were very similar. However, the phenol-sulfuric method resulted in carbohydrate levels approximately 45% higher than the other three analytical methods.

In all seven comparisons of freeze-dried vs. heat-dried tissue the heat-drying process showed lower carbohydrate levels. The loss was significant in four of the comparisons.

Table 1.--Percent carbohydrates in Merion Kentucky bluegrass leaves as determined by three extraction and four analysis procedures on freeze-dried and heat-dried tissue.

Extraction procedure	Carbohydrate analysis technique			
	Anthrone	Phenol-sulfuric	Nelson's arsenomolybdate	Shaeffer-Somogyi
20 C Water				
Freeze-dried	18.13*	26.94**	19.60**	
Heat-dried	16.90	24.02	17.79	
100 C Water				
Freeze-dried	17.61**	26.57	18.47	
Heat-dried	16.43	26.05	18.01	
Modified Weinmann				
Freeze-dried				18.26
Heat-dried				17.16

**Significant P<.01 comparing freeze-drying vs. heat-drying.

*Significant P<.05 comparing freeze-drying vs. heat-drying.

The same extraction and analysis comparisons were made on samples of Toronto creeping bentgrass leaf tissue. Percentage carbohydrates extracted by 20 C water, 100 C water, and the enzyme method were variable with no definite trends apparent (Table 2). The phenol-sulfuric acid analytical method yielded carbohydrate values approximately 80% higher than the other three techniques. Freeze-dried tissue contained significantly higher carbohydrate levels than heat-dried tissue in five of the seven comparisons.

No consistent difference existed in the extraction of carbohydrates from Tifgreen bermudagrass with either 20 C or 100 C water (Table 3). Approximately twice as much carbohydrate was extracted by the Modified Weinmann method. The results of the anthrone and Nelson's arsenomolybdate analysis procedure were similar, while the phenol-sulfuric acid method was again higher. The Shaeffer-Somogyi analysis method was over twice as high as water-soluble carbohydrates (except phenol-sulfuric analysis) because of the greater quantity of carbohydrates extracted from bermudagrass by the enzyme method. No significant difference in carbohydrate content was observed between freeze-dried and heat-dried bermudagrass leaf tissue.

Factors Affecting Carbohydrate Levels

Cutting Height

Merion Kentucky bluegrass was grown at 5- and 2.5-cm cutting heights at both 20 and 35 C temperatures and

Table 2.--Percent carbohydrates in Toronto creeping bentgrass leaves as determined by three extraction and four analysis procedures on freeze-dried and heat-dried tissue.

Extraction procedure	Carbohydrate analysis technique			
	Anthrone	Phenol-sulfuric	Nelson's arsenomolybdate	Shaeffer-Somogyi
20 C Water				
Freeze-dried	8.03**	14.74**	8.37*	
Heat-dried	7.11	11.88	6.84	
100 C Water				
Freeze-dried	7.93	14.97	8.07**	
Heat-dried	7.74	14.04	6.87	
Modified Weinmann				
Freeze-dried				8.11*
Heat-dried				5.43

**Significant P<.01 comparing freeze-drying vs. heat-drying.

*Significant P<.05 comparing freeze-drying vs. heat-drying.

Table 3.--Percent carbohydrates in Tifgreen bermudagrass leaves as determined by three extraction and four analysis procedures on freeze-dried and heat-dried tissue.

Extraction procedure	Carbohydrate analysis technique			
	Anthrone	Phenol-sulfuric	Nelson's arsenomolybdate	Shaeffer-Somogyi
20 C Water				
Freeze-dried	3.97	5.11	2.94	
Heat-dried	3.14	5.00	2.94	
100 C Water				
Freeze-dried	3.71	7.00	3.08	
Heat-dried	3.20	7.16	3.50	
Modified Weinmann				
Freeze-dried				8.09
Heat-dried				8.20

Note: N.S. between freeze-drying and heat-drying.

harvested weekly. Percent carbohydrate content in the leaf tissue tended to be higher at a 5-cm cutting height than at 2.5 cm when grown at 20 C, and tended to be higher at 2.5 cm than at 5 cm when grown at 35 C (Table 4). In neither case were the differences significant on the fifth week, probably indicating an adjustment to the cutting height. The carbohydrate content increased with each sampling at a soil temperature of 35 C. A comparable increase in carbohydrate content with time was not observed at 20 C.

Cutting Frequency

Weekly and semiweekly cutting on a Merion Kentucky bluegrass turf had no significant effect on the carbohydrate levels in the leaf tissue when plants were grown at 20 C (Figure 1).

Delayed Drying

A comparison of the percent carbohydrates in Merion Kentucky bluegrass when (a) grown at 20 vs. 35 C, (b) freeze-dried and heat-dried, and (c) the drying procedure delayed after sampling for 0, 15, 30, 60, and 120 minutes is shown in Table 5. The carbohydrate content in Merion Kentucky bluegrass leaves decreased as the time between sampling and drying was increased. Significant carbohydrate losses had occurred in all treatments when sample drying was delayed for 1 hour. The loss of carbohydrates for the four treatments ranged from 0.74 to 1.59% when drying was delayed for

Table 4.--The effect of cutting height on the water-soluble carbohydrate level of Merion Kentucky bluegrass leaves.

Cutting height (cm)	Soil temperature (C)	Percent carbohydrates				
		Time in weeks				
		1*	2	3	4	5
5.0	20	14.73**	10.39**	8.36	9.04**	9.15
2.5	20	10.16	7.06	7.51	7.51	8.19
5.0	35	10.96**	9.49	10.73	11.77	12.82
2.5	35	8.25	10.28	10.90	12.84**	13.28

*All grown at 20 C for first week.

**Significant P<.01 comparing the two cutting heights within a temperature.

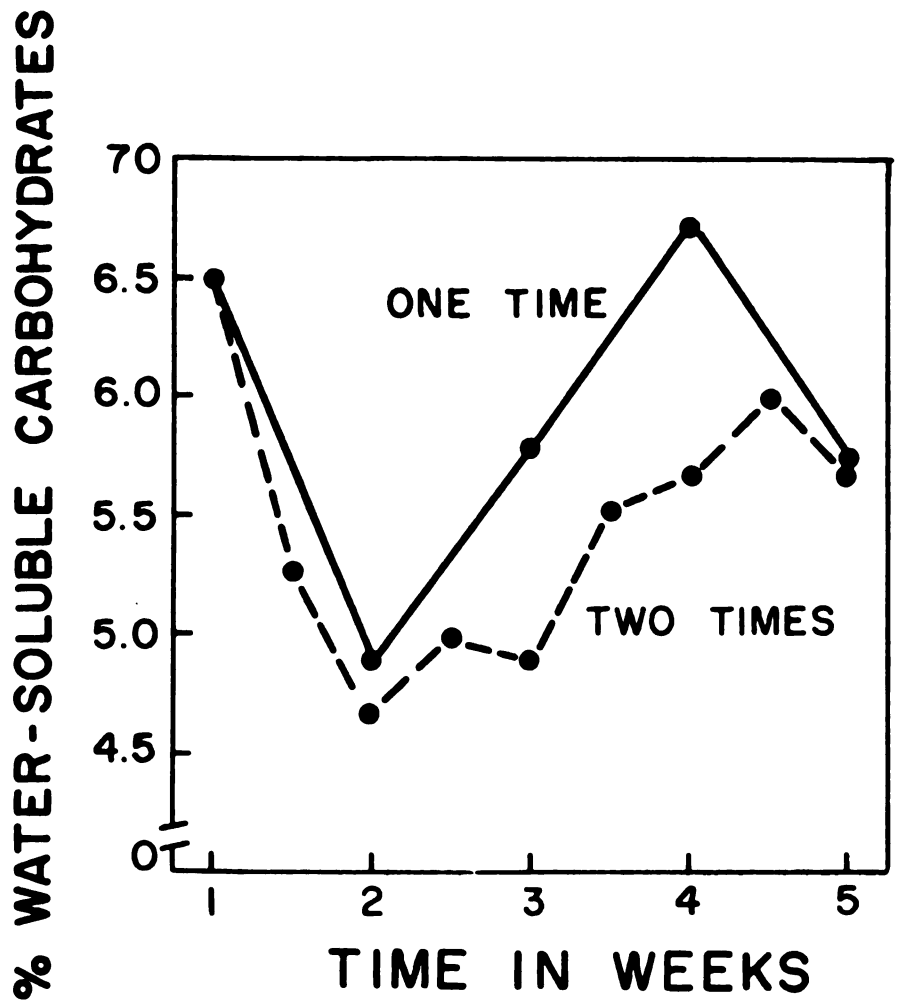

Figure 1.--Percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves cut one time vs. two times per week.

Table 5.--Comparisons of carbohydrate levels in Merion Kentucky bluegrass leaves grown at 20 and 35 C when drying was delayed 0, 15, 30, 60, and 120 minutes from the time of sampling.

Minutes from sampling to the initiation of sample drying	Percent carbohydrates*			
	Freeze-dried		Heat-dried	
	20 C	35 C	20 C	35 C
0	11.23a	10.10a	11.08a	9.52a
15	10.53 b	10.08a	10.64a	9.24a
30	10.71 b	9.82a	10.20ab	8.80 b
60	10.01 c	9.34 b	9.38 b	8.34 c
120	9.64 c	9.11 b	9.58 b	8.78 b

*Means within a column followed by the same letter are not significantly different at the .05 level using Tukey's multiple comparison test.

2 hours. Carbohydrate losses in tissue grown at 35 C were not significantly greater than the reduction in carbohydrates due to delayed drying in tissue grown at 20 C. Heat-drying in this experiment did not significantly reduce the carbohydrate content of the leaf tissue when compared to freeze-drying.

Diurnal Fluctuation

The diurnal fluctuation of water-soluble and 85% ethanol-soluble carbohydrate levels in Merion Kentucky bluegrass leaves during a 14-hour photoperiod is presented in Figure 2 and Table 6. The diurnal variation in water-soluble and 85% ethanol-soluble carbohydrate levels was very similar. Water-soluble carbohydrate levels increased from 4.37% at the start of the photoperiod to 7.06% 2 hours later and peaked at 13.84% at the end of a 14-hour day. The carbohydrate level at the end of the 10-hour dark period had decreased to 4.26%, which was similar to the starting level on the previous day.

Effects of Temperature on the Carbohydrate Levels

The temperatures discussed in the following experiments are soil temperatures rather than air temperatures. Air temperatures varied vertically within the turf microenvironment and from one side of controlled climate chambers to the other side. The microenvironment and leaf

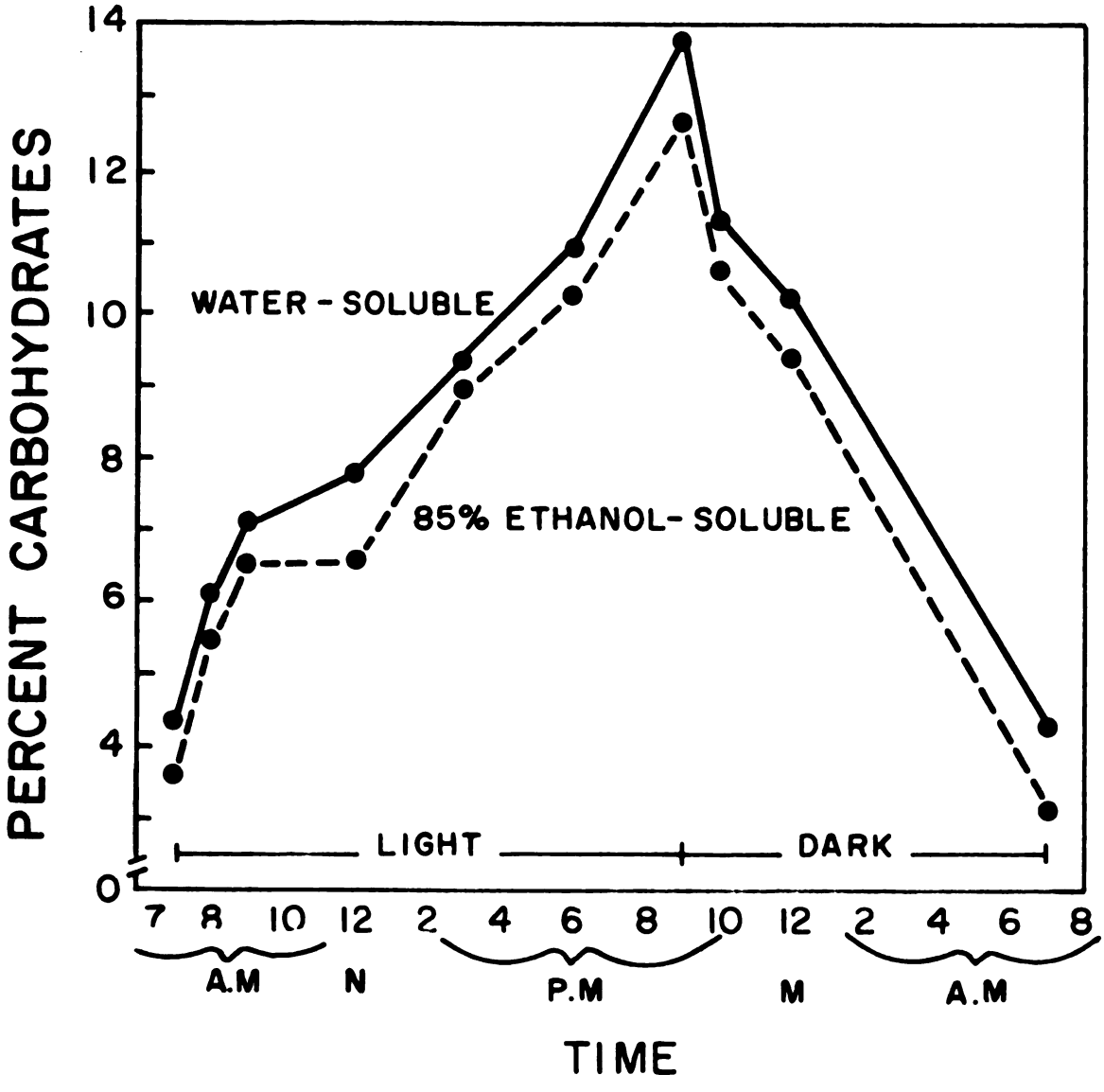

Figure 2.--Daily fluctuation of water-soluble and 85% ethanol-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at a 20 C soil temperature.

Table 6.--Diurnal fluctuation of water-soluble and 85% ethanol-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at a 20 C soil temperature.

Lights		Percent carbohydrates*	
Hours on	Hours off	Water-soluble	85% ethanol-soluble
	10	4.37a	3.59a
1		6.11 b	5.50 b
2		7.06 b	6.53 b
5		7.75 bc	6.55 b
8		9.44 cd	8.90 c
11		10.87 de	10.25 cd
14		13.84 f	12.70 e
	1	11.35 e	10.58 d
	3	10.19 de	9.42 cd
	10	4.26a	3.11a

*Means within a column followed by the same letter are not significantly different at the .01 level using Tukey's multiple comparison test.

temperature ranges corresponding with the principal soil temperatures used in this study are listed in Appendix Table 1. The microenvironment temperature frequently varied at least 4 C within a 35- x 50-cm sod piece. Soil temperatures could be maintained very near the programmed temperature.

Carbohydrate Levels at Four Temperatures

Dry matter production and water-soluble carbohydrate levels of Merion Kentucky bluegrass varied with changes in temperature (Figure 3). Maximum shoot growth and minimum carbohydrate levels occurred at a soil temperature of 25 C. Growth decreased slightly at 30 C while carbohydrates increased slightly. The first week at 35 C resulted in a 42% reduction in growth compared to growth at 30 C. Carbohydrates increased from 6.1 to 8.8% at the same sampling. This response for both growth and percent carbohydrates continued to an even greater extent during the second week at 35 C. Except for the second harvest, carbohydrate levels and growth were inversely related.

Carbohydrate Levels at 20 vs. 35 C

Further evaluations of the effects of optimal and supraoptimal temperatures on the carbohydrate levels were initiated. Turfs were grown at soil temperatures of 20 and 35 C, harvests were made weekly from several studies,

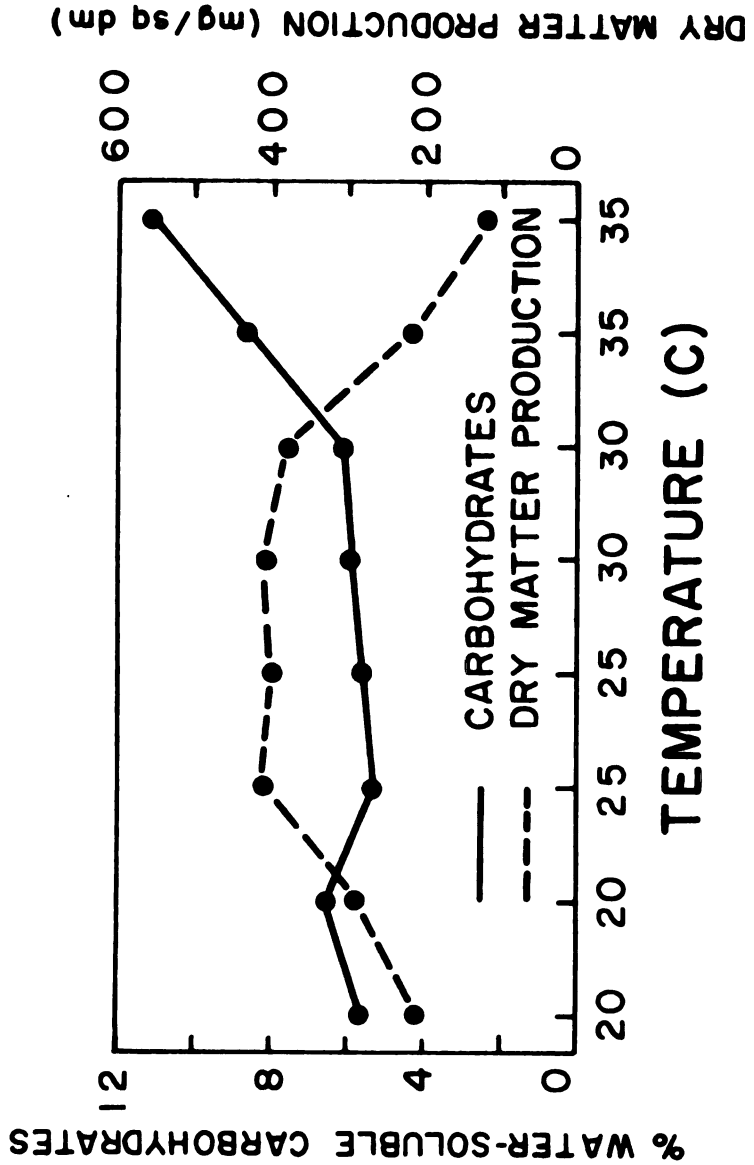

Figure 3.--Water-soluble carbohydrates and dry matter production of Merion Kentucky bluegrass grown for 2 weeks at each of four soil temperatures.

and water-soluble carbohydrate levels were determined (Figure 4 and Table 7). Carbohydrate levels were higher in plants grown at 35 C than at 20 C, with a difference of 3.8% the first week, and 8.8% the fifth week. Percent carbohydrates in Merion Kentucky bluegrass leaves grown at these two temperatures were significantly different for eight consecutive weeks. After harvesting the turf on the eighth week, the 35 C chamber was adjusted to 20 C. Carbohydrate levels of the 35 C treated turfs decreased to the same level as the turf from the 20 C chamber during the next 4 weeks at 20 C. Dry matter production could not be correlated to the carbohydrate levels on a weekly basis. For the entire experiment however, carbohydrates increased as the shoot growth decreased. The average shoot growth rate for the 8-week period was 451 and 111 mg $dm^{-2}week^{-1}$ at 20 and 35 C, respectively, while the carbohydrate content averaged 9.1 and 15.2%, respectively. The results of a preliminary comparison of carbohydrate levels in Merion Kentucky bluegrass leaves grown at 20 and 35 C are presented in Appendix Table 2. The results were consistent with the above experiment.

Diurnal Temperature Study

In a day-night temperature regime, carbohydrates were lower at 35-25 C than at 23-15 C (Figure 5). Carbohydrate levels were 24 and 20% during the first 2 weeks at 23-15 C. After 2 weeks at 35-25 C, the carbohydrate levels

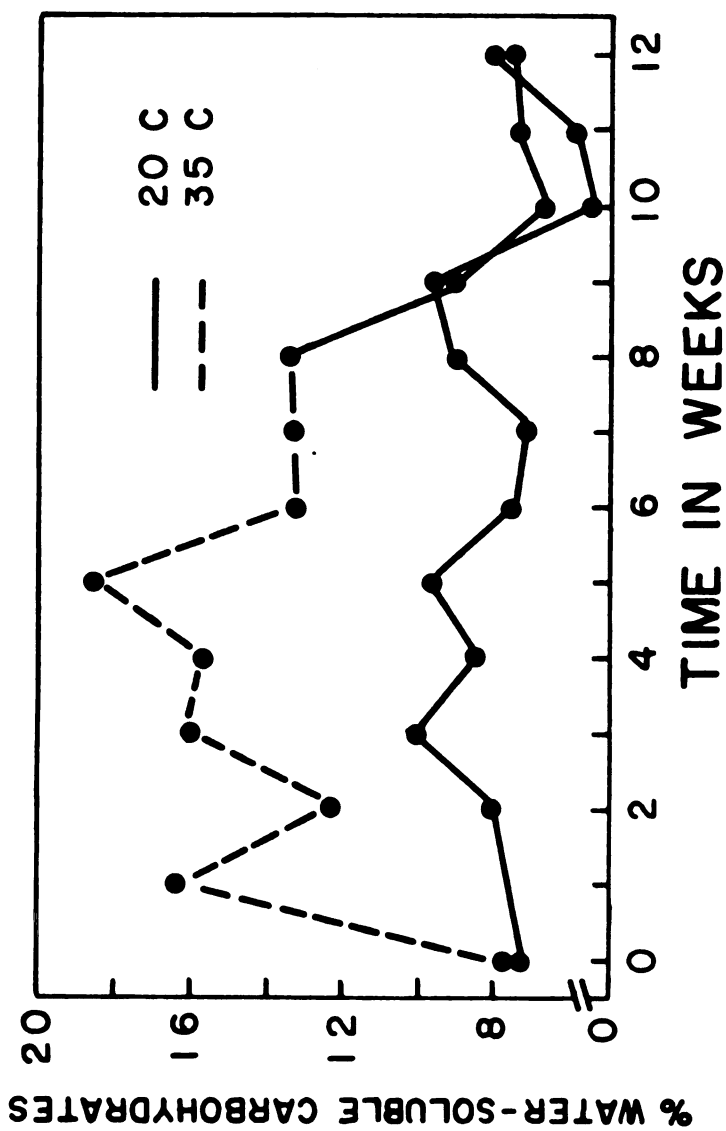

Figure 4.--Percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at 20 and 35 C soil temperatures.

Table 7.--Dry matter production and percent water-soluble carbohydrates of Merion Kentucky bluegrass grown at soil temperatures of 20 and 35 C.

Time in weeks	Shoot growth (mg dm^{-2} $week^{-1}$)		Water-soluble carbohydrates (%)	
	20 C	35 C	20 C	35 C
0			7.7	7.9
1	399	314	12.6	16.4**
2	382	81	8.0	12.2**
3	362	105	10.1	16.0**
4	413	78	8.6	15.8**
5	470	105	9.8	18.6**
6	558	74	7.6	13.2**
7	532	58	7.1	13.4**
8	491	70	9.0	15.7**
9	532	*147	9.5	* 9.2
10	568	*310	5.4	* 6.8
11	473	*387	6.1	* 7.5
12	411	*360	8.1	* 7.8

*Changed to a growth temperature of 20 C.

**Significant $P<.01$ comparing percent carbohydrates at 35 C to 20 C.

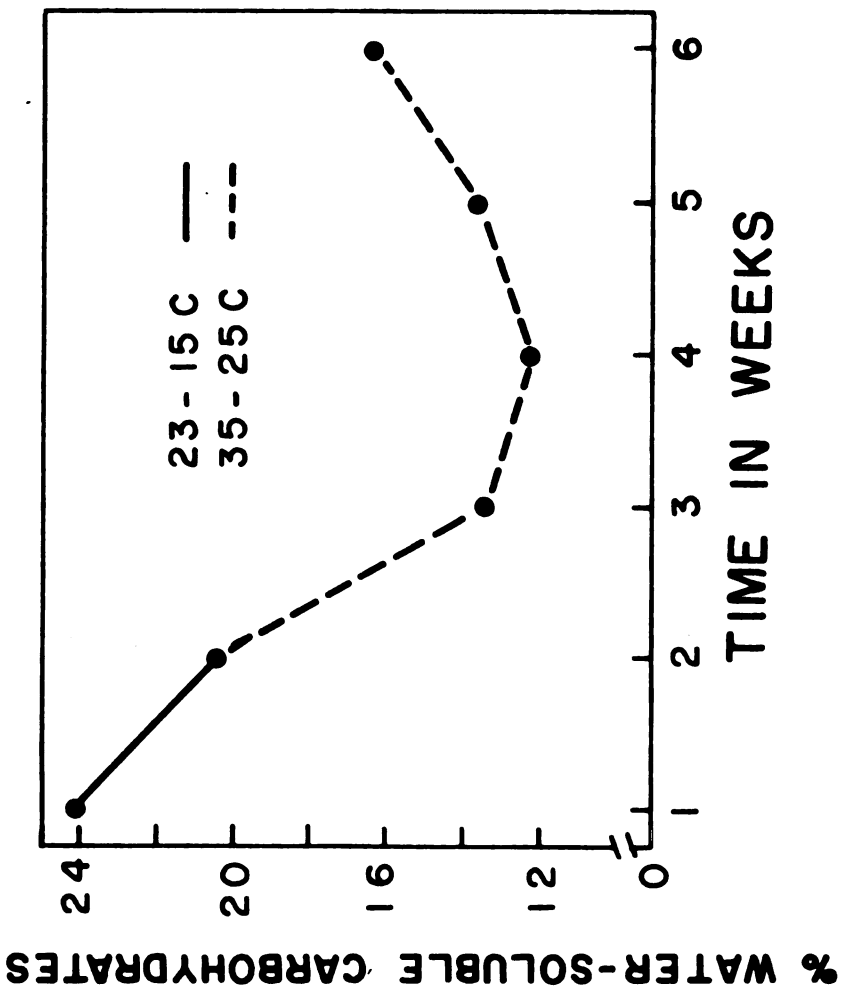

Figure 5.--Percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at day-night temperature regimes of 23-15 and 35-25 C soil temperatures.

had decreased to 12%. Carhohydrates had increased to 16% at the conclusion of the experiment 2 weeks later.

Morning vs. Afternoon Sampling Comparisons

Results previously presented indicated a three fold increase in water-soluble carbohydrates during a 14-hour light period (Figure 2). In this study, Merion Kentucky bluegrass leaves grown at 20 C and sampled 2 and 10 hours following initiation of the photoperiod showed about a two fold increase in carbohydrates from the 2 to the 10 hour sampling time (Figure 6). After 4 weeks, one-half the sod was placed in a 35 C soil temperature regime. Carbohydrate levels of leaf tissue grown at 20 and 35 C and harvested 2 hours after initiation of the photoperiod were inversely related to those harvested 8 hours later. After 2 hours in the light, the 3-week averages showed the 35 C tissue to be higher in carbohydrates than the 20 C tissue by 1.3%, while the 20 C tissue was higher after 10 hours by 2.7%. A greater difference was apparent on specific sampling dates. The sample at 10 hours was significantly higher in carbohydrate content than the 2-hour sample in all cases (Table 8).

In a similar experiment, carbohydrates were about 2% higher in tissue from plants grown at 35 C at the end of the dark period, while at the end of an 11-hour day the carbohydrates were 3% lower (Figure 7). In this particular

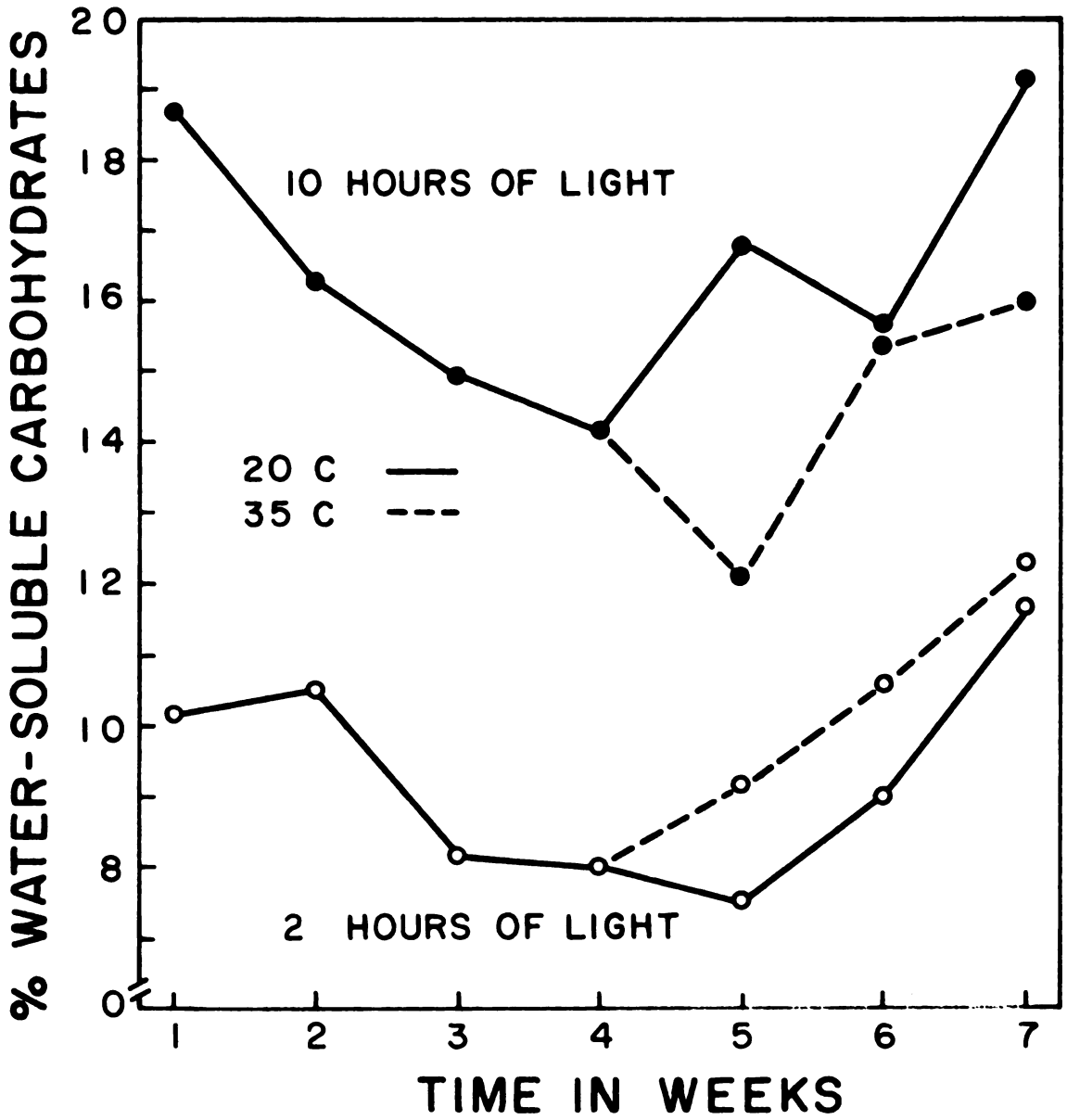

Figure 6.--Percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at 20 and 35 C soil temperatures and harvested 2 and 10 hours after initiation of the light period.

Table 8.--Comparison of water-soluble carbohydrate levels in Merion Kentucky bluegrass leaves harvested 2 and 10 hours after initiation of the light period at soil temperatures of 20 and 35 C.

Sampling time (Hours)	Soil temperature (C)	Percent carbohydrates						
		Week						
		1	2	3	4	5	6	7
2	20	10.30	10.96	8.61	8.05	7.49	8.97	11.65
10	20	19.17**	16.09**	15.71**	14.69**	16.75**	15.70**	19.20**
	After week 4							
2	35	10.05	10.01	7.79	7.91	9.18	10.55	12.26
10	35	18.16**	16.53**	14.19**	13.74**	12.05**	15.43**	15.97**

**Significant P<.01 comparing the two sampling times within a temperature.

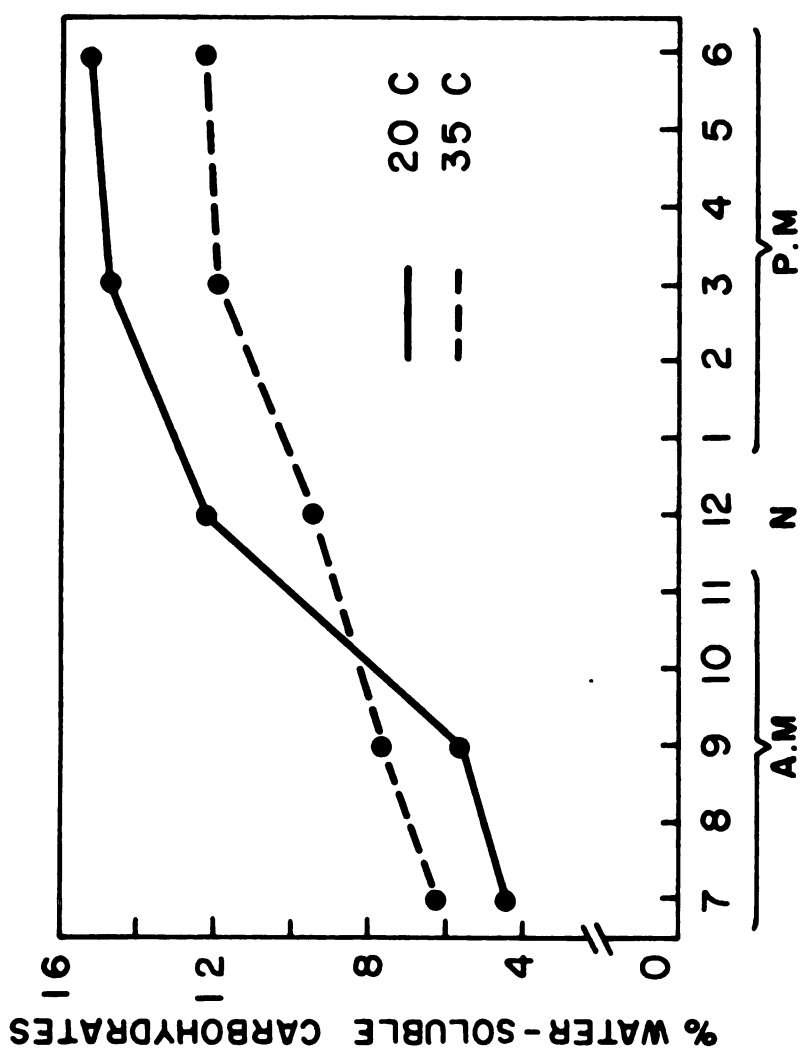

Figure 7.--Daily fluctuation of water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at 20 and 35 C soil temperatures.

experiment, the leaf tissue from plants grown at a soil temperature of 20 and 35 C had equal carbohydrate levels between 2 and 5 hours after initiation of the light period.

Carbohydrate Content in Leaves, Stems, and Roots

Total dry matter production by Merion Kentucky bluegrass grown at soil temperatures of 20 and 35 C was highest for the leaves, followed by the stems and then the roots (Table 9). The water-soluble carbohydrate content for the first 4 weeks was higher in leaves, stems, and roots grown at 20 C than at 35 C. By the fifth week the leaf carbohydrate content was comparable at 20 and 35 C, while carbohydrate levels were higher in stems at 35 C and roots at 20 C. The carbohydrate level of the leaves and stems was about equal at 20 and 35 C. During a 5-week period at 20 C, the carbohydrate level increased in the leaves and stems initially and then decreased during the last two samplings (Figure 8). At 35 C, carbohydrate levels in the leaves and stems decreased and then increased to their former level. Root carbohydrate levels at the first sampling were about one-half that of leaves and stems. Carbohydrate levels decreased initially at both 20 and 35 C. However, by the end of 5 weeks the carbohydrates were again approaching the level of the first sampling.

Table 9.--Dry matter production (g/pot) and percent carbohydrates (%CH_2O) of Merion Kentucky bluegrass leaves, stems, and roots grown at soil temperatures of 20 and 35 C.

Time in weeks	Growth temperature (C)	Leaves		Stems		Roots	
		g/pot	%CH_2O	g/pot	%CH_2O	g/pot	%CH_2O
0		3.7	23.4	2.0	23.6	1.5	10.7
1	20	5.5	21.9**	3.7	23.6**	1.9	7.6**
	35	3.4	7.2	2.7	20.8	1.1	2.2
2	20	5.5	30.6**	4.0	27.6**	1.9	6.7**
	35	4.7	11.0	2.8	18.6	0.9	1.7
3	20	5.3	30.3**	5.1	30.8**	3.4	9.7**
	35	5.2	14.7	3.1	20.8	1.0	1.8
4	20	5.3	28.4**	6.0	28.3**	3.9	9.2**
	35	4.6	18.6	2.6	23.1	0.6	2.3
5	20	8.1	23.4	5.9	21.5	4.5	10.6**
	35	5.4	23.9	4.3	26.1**	1.1	8.2

**Significant $P<.01$ comparing %CH_2O at 20 C vs. 35 C.

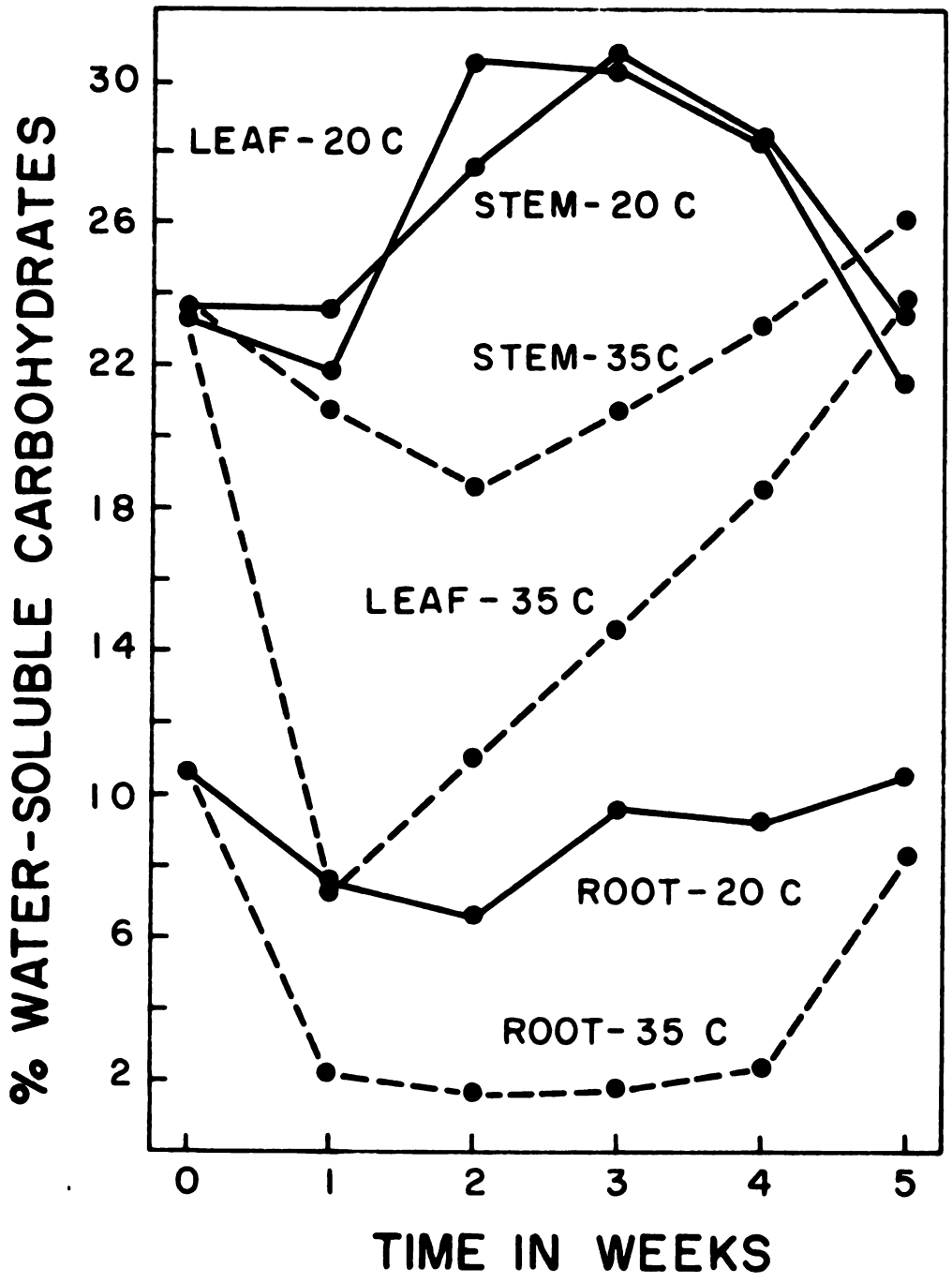

Figure 8.--Percent water-soluble carbohydrates in leaves, stems, and roots of Merion Kentucky bluegrass grown at 20 and 35 C soil temperatures.

Photoperiod Study

Dry-matter production was measured in Merion Kentucky bluegrass leaves grown at 20 and 35 C and at photoperiods of 10, 12, 14, 16, and 18 hours (Table 10). Average shoot growth increased as the duration of light increased, except for the 14-hour photoperiod. Shoot growth at 35 C averaged only about 10% of that observed at 20 C after the fifth week.

Carbohydrate levels were higher at 35 C than at 20 C for all five photoperiods except at 16 hours for the first 3 weeks and at 18 hours where they were equal at the fifth week.(Figure 9). The carbohydrate levels for all photoperiods at 20 C were between 6.1% and 7.9% after 5 weeks except the 18-hour photoperiod which was 13.1%, but was also declining. Comparing the first and last samplings, the general trend showed carbohydrate levels being relatively stable at 20 C and increasing at 35 C. Carbohydrate levels at a 35 C growth temperature increased more at short photoperiods than longer photoperiods.

The average carbohydrate level in leaves of Merion Kentucky bluegrass grown at a 20 C soil temperature decreased significantly with a shorter light exposure, while no differences occurred at 35 C (Table 11).

The average carbohydrate level in Merion Kentucky bluegrass leaves was significantly higher at a 35 C growth temperature compared to 20 C at all photoperiods except the 18-hour regime where the reverse was true (Table 12).

Table 10.--Dry matter production of Merion Kentucky bluegrass grown at soil temperatures of 20 and 35 C and five photoperiods.

Photoperiod (hours)	Soil temperature (C)	Dry matter (mg dm^{-2})					
		Time in weeks					
		1*	2	3	4	5	Avg.
10	20	509	410	405	432	443	423
	35	709	294	117	55	50	129
12	20	715	587	498	571	565	555
	35	648	327	188	117	67	175
14	20	526	471	487	465	382	451
	35	492	294	161	67	67	147
16	20	737	620	604	665	598	622
	35	797	515	227	100	61	226
18	20	753	720	704	648	665	684
	35	676	465	277	117	67	232

*All grown at 20 C for first week.

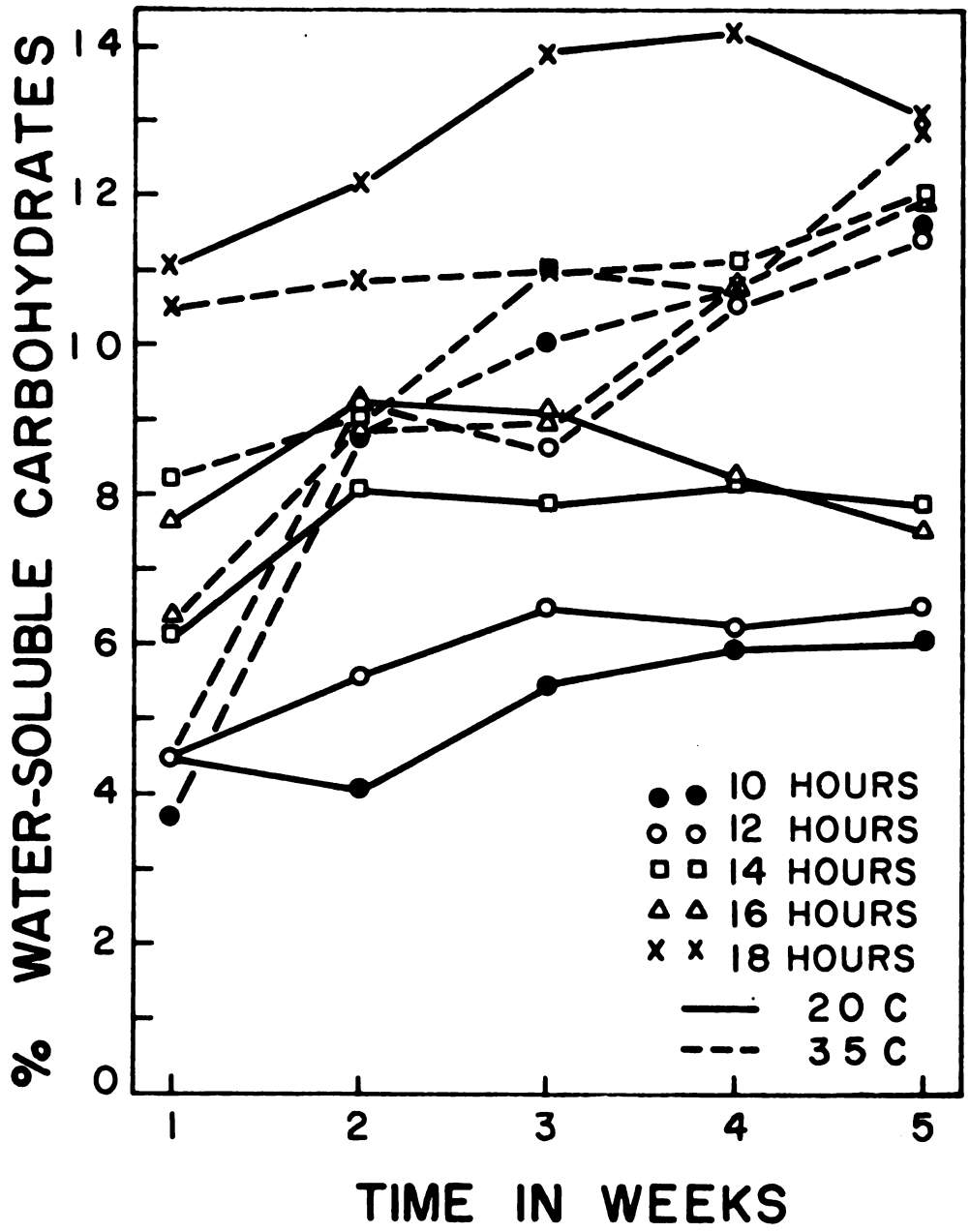

Figure 9.--Percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at 20 and 35 C soil temperatures and five photoperiods.

Table 11.--Effect of photoperiod on water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at two temperatures (average of four weekly samplings).

Photoperiod (hours)	Percent carbohydrates*	
	Soil temperature	
	20 C	35 C
18	13.3a	10.7a
16	8.6 b	10.2a
14	8.0 b	10.9a
12	6.3 bc	10.0a
10	5.4 c	10.4a

*Means within a column followed by the same letter are not significantly different at the .01 probability level using Tukey's test.

Table 12.--Effect of temperature on water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at five photoperiods (average of four weekly samplings).

Growth temperature(C)	Photoperiod (hours)				
	18	16	14	12	10
20	13.3**	8.6	8.0	6.3	5.4
35	10.7	10.2*	10.9**	10.0**	10.4**

**Means are significantly different at .01 probability level comparing the two growth temperatures within a photoperiod.

Photosynthetic and Respiratory Rate Study

Merion Kentucky bluegrass was conditioned at soil temperatures of 20 and 35 C in controlled environment chambers for 1 week. Photosynthetic and respiratory rates were measured at 20, 25, 30, 35, and 40 C (Figure 10). Respiratory rates continued to increase through 40 C. Photosynthesis increased from 20 to 25 C, but decreased from 25 through 40 C. The magnitude of photosynthesis was still greater than respiration at 40 C. The Kentucky bluegrass conditioned at 20 C showed a higher respiration rate at all test temperatures. The same was true for photosynthesis at all test temperatures except 40 C, where the photosynthetic rate in the tissue conditioned at 35 C did not decrease as rapidly; consequently, photosynthesis remained higher than for the 20 C tissue.

In a subsequent study where the turf was conditioned at 20 and 35 C soil temperatures for 4 weeks, respiration again increased through 40 C in both instances (Figure 11). Respiration in the turf conditioned at 20 C was about two times greater than respiration in the turf conditioned at 35 C. Respiratory rates of turfs grown at 20 C exceeded photosynthetic rates between test temperatures of 25 and 30 C. In turfs conditioned at 35 C respiration exceeded photosynthesis between 30 and 35 C. The photosynthetic rate decreased rapidly at high temperatures after an initial increase from 20 to 25 C. The rate of

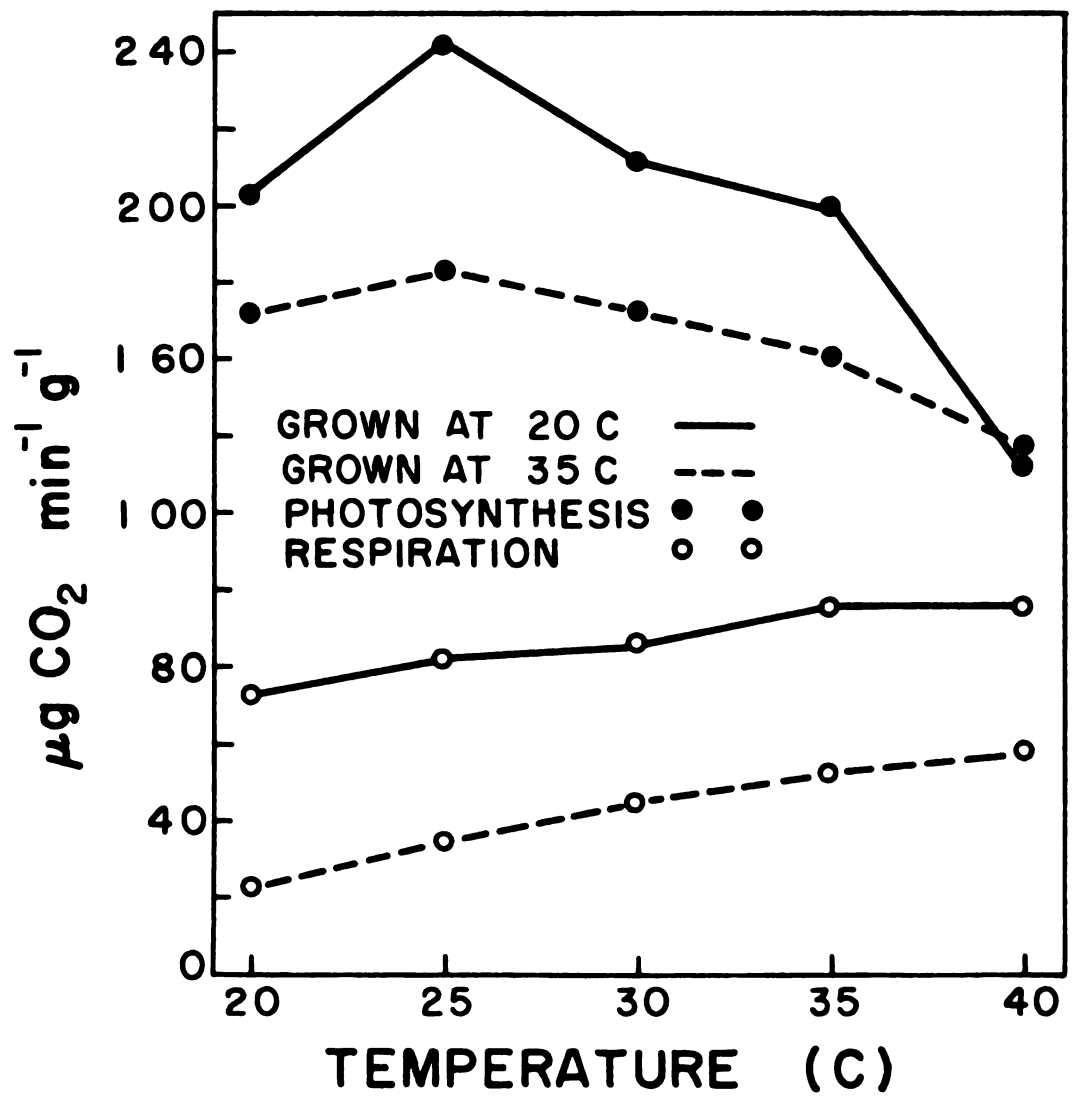

Figure 10.--Photosynthetic and respiratory rates of Merion Kentucky bluegrass when grown at a 20 and 35 C soil temperature for 1 week.

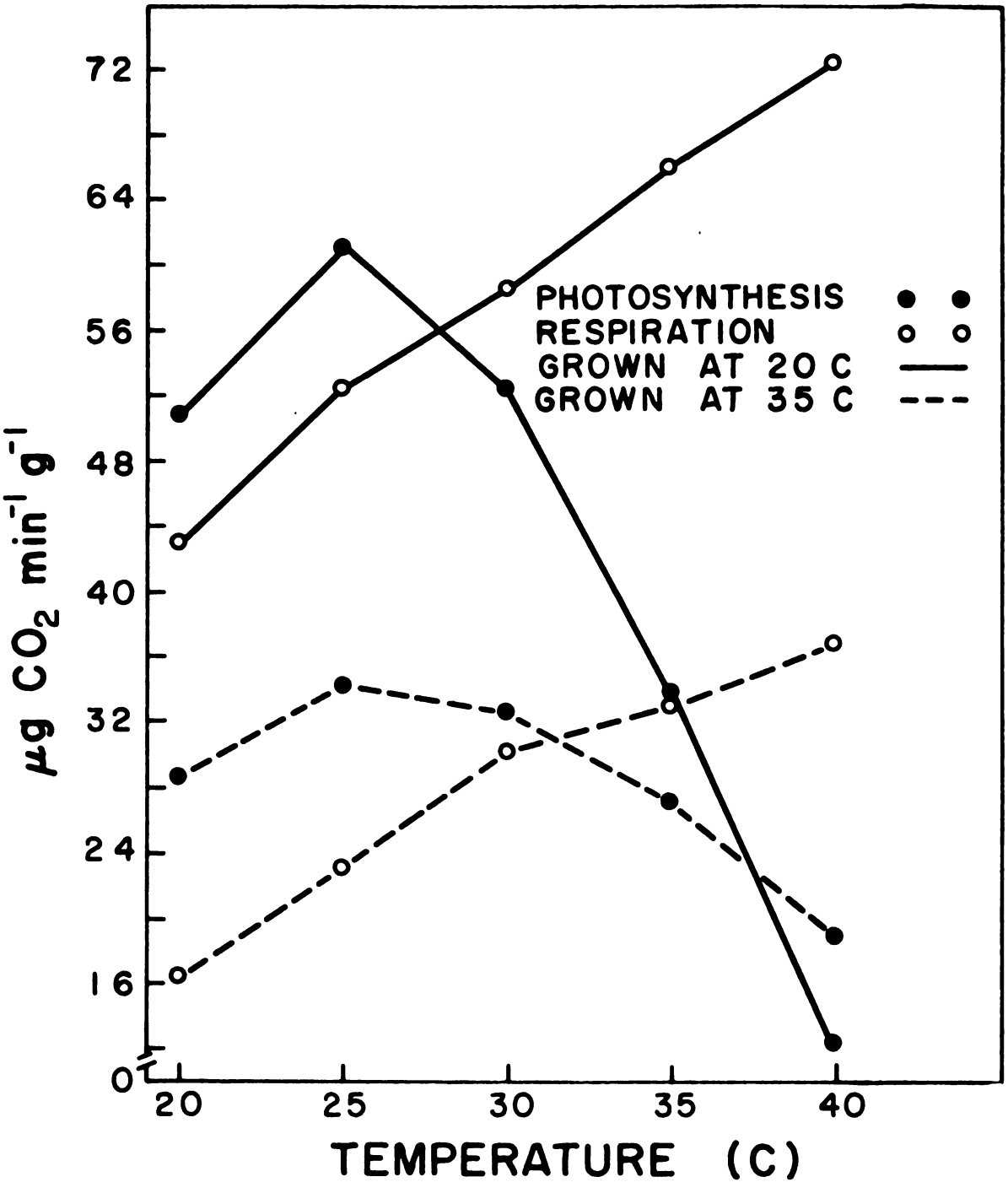

Figure 11.--Photosynthetic and respiratory rates of Merion Kentucky bluegrass when grown at a 20 and 35 C soil temperature for 4 weeks.

photosynthesis in turf grown at 20 C dropped below the rate in turf grown at 35 C at the 40 C test temperature. Respiration exceeded photosynthesis in the 20 C turf by about six fold at the 40 C test temperature, but by less than two fold in the 35 C turf.

DISCUSSION

Three carbohydrate extraction procedures, 20 C water, 100 C water, and a takadiastase enzyme, were utilized in extracting total nonstructural carbohydrate (TNC) from Merion Kentucky bluegrass, Toronto creeping bentgrass, and Tifgreen bermudagrass. While some variation occurred between procedures, the magnitude of difference was small between the two cool-season grasses, Kentucky bluegrass and creeping bentgrass. The three extraction methods were adequate and approximately equal for bluegrass and bentgrass, therefore the choice of a method may be decided on the basis of personal preference or convenience. The 20 C water extraction method was preferred over the 100 C water extraction method. The enzyme method was more time-consuming, laborious, and in the experience of the author, not as repeatable among replications. However, the enzyme method was more useful for carbohydrate determinations of a warm-season grass that accumulates starch, such as bermudagrass, since starch is not extracted by water. In the previously reported experiments with bermudagrass, TNC was twice as high from enzyme than

from water extraction, presumably due to the starch content. For comparative purposes, it may be desirable to use the enzyme extraction for TNC when both fructosan storing and starch storing grasses are included in the same experiment.

The anthrone and arsenomolybdate colorimetric and the Shaeffer-Somogyi titration techniques for carbohydrate analysis all produced comparable results. Data from different experiments or different laboratories using any of these three analysis techniques could be compared, allowing for a small degree of error. The phenol-sulfuric method resulted in TNC values 40 to 80% higher than the other three analysis techniques. This technique is very sensitive to any lint or cellulosic contaminant, although care was exercised to avoid this. The explanation for the higher values in this investigation is not available. The arsenomolybdate and Shaeffer-Somogyi methods are more time-consuming than the other two. The anthrone procedure proved to be rapid, convenient, and repeatable, and was utilized throughout the remainder of this investigation.

Drying fresh plant tissue for TNC analysis presents several problems. Destruction of carbohydrates during heat-drying may occur at a very high temperature while respirational losses may occur if the temperature is not high enough to denature the enzymes. Losses of carbohydrates are less in freeze-drying, but interconversions may occur if the temperature is not low enough. In this

investigation the TNC levels were generally higher in plant tissue that was freeze-dried. The differences were not greater than 3% in any instance. Heat-drying is usually more convenient and is probably comparable, within a few percentage points, among laboratories.

Generally metabolic processes are more rapidly terminated after sampling by freeze-drying than by heat-drying, since dry ice can be used immediately in the former instance, while there may be a delay in getting the tissue to the drying oven in the latter. Assuming more rapid metabolic rates at higher temperatures, it is conceivable that greater TNC losses could occur in tissue grown at a high temperature than at a low temperature if initiation of the drying process were delayed. However, this was shown not to be the case in this study (Table 5). Although heat-drying and a delay in drying by either method resulted in the loss of carbohydrates, there was no greater percentage loss in tissue grown at 35 C compared to 20 C. Therefore, it would probably not be a significant factor in comparing carbohydrate levels at several growing temperatures.

The effect of 5- and 2.5-cm cutting heights on water-soluble carbohydrate levels in Merion Kentucky bluegrass was investigated at 20 and 35 C. Carbohydrate levels were higher at a 5-cm cutting height when grown at 20 C, and at 2.5 cm when grown at 35 C. However, carbohydrate levels were higher at 35 C than at 20 C at both cutting

heights after the second week. No shift in the higher carbohydrate level from one temperature to the next occurred due to cutting height. Cutting height differentials do not appear to explain the variation among laboratories regarding the temperature at which highest carbohydrate levels occur. However, the specific carbohydrate level could be affected by the cutting height.

The foregoing conclusion was also true for cutting frequency. A maximum 1% variation occurred in carbohydrate levels of Merion Kentucky bluegrass leaves cut either weekly or semiweekly. Infrequent defoliations on forage grasses may significantly alter carbohydrate levels, but apparently not with turfgrasses cut on a regular basis.

Carbohydrate production is the result of a series of reactions that occur in the green plant. Carbon dioxide is reduced in photosynthesis to provide carbon skeletons for intermediary metabolism, cell wall synthesis, and carbohydrate reserves. Growth generally decreases after carbohydrates are depleted at a given temperature. This has been suggested in some turfgrass literature as the causal mechanism for reduction of growth at supraoptimal temperatures (57).

In this investigation, carbohydrate level and growth of cool season turfgrasses were inversely related as the temperature was increased from 20 to 35 C. As growth increased, the carbohydrate level decreased. In six

separate studies comparing carbohydrate levels in leaf tissue grown at 20 and 35 C, carbohydrate levels were always higher at 35 C, while maximum shoot growth occurred at 20 C. This does not support the theory that lack of shoot growth at supraoptimal temperatures (35 C in this study) is caused by exhaustion of carbohydrates, or even that they are approaching threshold levels.

Carbohydrate levels were also assayed in the stems and roots to ascertain whether leaf carbohydrate levels remained high simply because of transport from and depletion of levels in the stems and roots. Carbohydrate levels in the stems and roots decreased initially at 35 C. The short term decrease in carbohydrate levels was possibly due to an initial stimulation in shoot growth or the fact that it was not a mature turf. Carbohydrate levels recovered after several weeks to a level comparable with those found in plants grown at 20 C. The increase in carbohydrate levels in leaves at 35 C was apparently due to some factor other than translocation.

When Merion Kentucky bluegrass was grown at 35 C for a period of time and then transferred to 20 C, growth increased while the carbohydrate level decreased to the same level as the turf growing at 20 C for the entire experiment. Conclusions in the preceding paragraphs support the contention that carbohydrate levels are adequate in

Merion Kentucky bluegrass grown at 35 C, but for some reason are not being respired or utilized for growth.

A hypothesis could be formulated that carbohydrate levels of 10 or 15% in tissues growing at 35 C are already too low, and that they cannot be depleted or utilized to any lower level. This is refuted by Appendix Table 3 where carbohydrate values were lowered from 9.7% to 5.7% by a 2-hour day. In addition, carbohydrate levels at 35 C decreased from 10.5% to 2.5% during a 48-hour night. Had the initial level been a threshold carbohydrate level, neither a reduction in carbohydrates nor an elongation of the leaf tissue would have occurred. Both situations occurred, however. Thus, threshold carbohydrate levels had not been obtained.

Carbohydrate levels at any given temperature are influenced by the previous conditions in which the turf was grown. Such variables as a seedling turf or established turf, season of the year, length of time in the greenhouse, preconditioning time in the controlled climate chamber, etc., all influence the carbohydrate level. Therefore, it is extremely important to measure carbohydrates at all variables (e.g. two temperatures) during an entire experiment so that valid comparisons can be made.

Merion Kentucky bluegrass was grown at a day-night temperature of 23-15 C followed by 35-25 C and a photoperiod of 16 hours to repeat a previous study reported in

the literature (57). Carbohydrate levels decreased 7% during the first week at the higher temperature, but instead of continuing toward exhaustion, they increased the third and fourth week at the 35-25 C day-night temperature (Figure 5). The importance of the preceding paragraph is illustrated by the fact that carbohydrates at a day-night temperature of 23-15 C had already decreased 4% from week one to two, and since the lower temperature was not continued, no accurate comparisons were possible.

Another factor significantly affecting carbohydrate levels in plant tissue is the time of sampling in relation to the day-night regime. In this investigation the diurnal fluctuation of water-soluble carbohydrates at 20 C varied three-fold from the lowest to highest level. This was a greater variation than has commonly been reported in the literature (26, 28, 32). Most of this unusually large fluctuation occurred due to the increase in monosaccharides produced during the day by photosynthesis and transported out of the leaf or utilized at night through respiration.

Because of the diurnal fluctuation in carbohydrate content, an experiment was initiated to compare carbohydrate levels in the morning and evening in Merion Kentucky bluegrass grown at 20 and 35 C. From a morning to evening sampling, leaf carbohydrate levels increased almost 200% at 20 C, and only 35% at 35 C. While carbohydrate levels were higher at 35 C in the morning sampling, the comparison

was reversed in the evening sampling when carbohydrate levels were higher at 20 C.

The explanation is evident from the photosynthesis and respiration experiments (Figure 11). Both photosynthetic and respiratory rates of Merion Kentucky bluegrass preconditioned at 20 C are higher at 20 C than the rates when preconditioned at 35 C and tested at 35 C. Therefore, the Merion Kentucky bluegrass at 20 C will have a higher carbohydrate content at the end of the day, but because of the higher respiration rate will also have a lower carbohydrate level at the end of the night.

In attempting to ascertain the quantity of carbohydrates available for growth at an optimal and supraoptimal temperature, the time of sampling will completely alter the comparison. The reversal of results occurred between 2 and 5 hours into the photoperiod. The following question then, becomes crucial: Which of the sampling periods is more valid for investigating carbohydrate levels in relation to reduced growth at supraoptimal temperatures?

The hypothesis in question states that carbohydrate depletion or exhaustion is directly responsible for the drastic reduction of growth of Merion Kentucky bluegrass at 35 C. Therefore, determination of the optimum temperature for maximum carbohydrate production during the day is not as crucial as determining whether sufficient carbohydrates are available at supraoptimal temperatures for

respiration and growth. This can best be determined by monitoring carbohydrate levels immediately after the dark period since that is the time a carbohydrate deficit would most logically occur. This investigation has shown that carbohydrate reserves are not exhausted at the end of the night at 35 C; consequently, carbohydrate reserves are not the limiting factor for growth of Merion Kentucky bluegrass at supraoptimal temperatures.

The photoperiod experiment also supports this conclusion. All grasses were grown at 20 C during the first week and then half were changed to 35 C. For carbohydrate exhaustion to become apparent at the higher temperature, carbohydrate levels would be expected to decrease at all photoperiods, but more rapidly at the short photoperiods. Instead, carbohydrate levels increased at the higher temperature, and all five photoperiods had nearly the same levels after 4 weeks. This apparently indicates some block in carbohydrate utilization compared to the expected rate.

Preconditioning Merion Kentucky bluegrass for 1 week at 20 and 35 C resulted in lower rates of photosynthesis and respiration at five test temperatures for the 35 C turf. Apparent photosynthesis at test temperatures of 35 and 40 C was higher in turf conditioned at 35 C. Duff (13) also measured higher photosynthetic rates at 20, 30, and 40 C test temperatures in Toronto creeping

bentgrass grown at a 40-30 C day-night temperature regime compared to 20-10 C using Warburg manometric techniques. Some adaptation to the higher temperature occurred in both instances. After 4 weeks of preconditioning at 20 C, the rate of respiration exceeded photosynthesis at a test temperature of 30 C, while respiration exceeded photosynthesis at a 35 C test temperature for the turfs preconditioned at 35 C. The difference between respiratory and photosynthetic rates was much greater at 35 and 40 C in the grasses preconditioned at 20 C. The low photosynthetic rates after 4 weeks were probably caused by the low light intensities for this experiment in the controlled climate chambers.

This investigation indicates that reduced growth of Merion Kentucky bluegrass at supraoptimal temperatures is not caused by inadequate carbohydrate skeletons for plant metabolism. Differences between these results and results previously published (21, 44, 53, 56, 57, 61) supporting an opposite conclusion probably are not due to (a) plant harvesting and drying methods, (b) carbohydrate analysis procedures, (c) cutting height or frequency, (d) transport of carbohydrates from stems and roots to leaves, or (e) photoperiod, but may possibly be due to (a) morning vs. afternoon sampling, (b) initial carbohydrate levels in the plant tissue, or (c) a

relatively low supraoptimal temperature level. The primary causes of growth reduction at supraoptimal temperatures remain to be elucidated.

CONCLUSIONS

1. Three carbohydrate extraction methods, 20 C water, 100 C water, and Modified Weinmann, and three analysis procedures, anthrone, phenol-sulfuric, and Shaeffer-Somogyi, resulted in similar carbohydrate levels in Merion Kentucky bluegrass and Toronto creeping bentgrass.

2. Heat-drying plant tissue generally resulted in greater losses of carbohydrates than freeze-drying. A delay of 2 hours in initiating the drying process did not result in large carbohydrate losses.

3. A cutting height of 5 cm vs. 2.5 cm did not produce a shift in carbohydrate levels at 20 vs. 35 C.

4. Cutting frequency did not significantly alter leaf carbohydrate levels.

5. Water-soluble carbohydrates were consistently higher in Merion Kentucky bluegrass grown at a soil temperature of 35 C than at 20 C.

6. Dry-matter production of Merion Kentucky bluegrass decreased as temperature was increased above the optimum (25 C in this investigation) for growth.

7. Water-soluble carbohydrate levels increased more than three-fold on several sampling days. Greater fluctuations occurred at a growth temperature of 20 C than at 35 C; consequently, carbohydrates were higher at 35 C than at 20 C during the first several hours of the photoperiod, but lower at 35 C from 5 hours to the end of the photoperiod. Hence, the time of sampling may account for different results reported by several laboratories.

8. The higher carbohydrate levels in Merion Kentucky bluegrass leaves at 35 C are not due to a decrease in carbohydrate reserves in the stems or roots.

9. Respiration continued to increase from test temperatures of 20 to 40 C for Merion Kentucky bluegrass preconditioned at 20 and 35 C, but the rate was higher for plants grown at 20 C. Maximum photosynthesis occurred at 25 C, then decreased with increasing temperature. The decrease was less rapid for Merion Kentucky bluegrass preconditioned at 35 C. An adaptive mechanism was apparently operative.

10. Factors other than those investigated cause growth-reduction of Merion Kentucky bluegrass at supra-optimal temperatures. It cannot be attributed simply to a depletion of the reserve carbohydrates due to the rate of respiration exceeding photosynthesis over a period of time.

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APPENDIX

APPENDIX

Table A1.--Comparison of leaf temperature and microenvironment temperature to soil temperatures of 20 and 35 C in a controlled environment chamber.

Soil temperature (Bulb thermometer) (C)	Microenvironment temperature (Leeds & Northrup Potentiometer) (C)	Leaf temperature (Stoll-Hardy HL4 Radiometer) (C)
20	22-25	21-24
35	36-39	33-35

Table A2.--Preliminary study of percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves grown at 20 and 35 C soil temperatures.

Time in weeks	Percent carbohydrates	
	Growth temperature	
	20 C	35 C
1	5.8	9.8
2	4.0	9.0
3	5.6	13.0
4	7.5	14.0
5	6.3	13.2
6	5.2	16.0

Table A3.--Percent water-soluble carbohydrates in Merion Kentucky bluegrass leaves at the end of two different photoperiods and at several times during continuous darkness when grown at 35 C.

Treatment	Sampling time	Percent carbohydrates
14 hour day	After 10 hour night	9.7
2 hour day	After 22 hour night	5.7
14 hour day	After 10 hour night	10.5
24 hour night	After 24 hour night	4.5
36 hour night	After 36 hour night	3.7
48 hour night	After 48 hour night	2.5