ABSTRACT

THE EFFECT OF HEAT STRESS ON ASPECTS OF CARBOHYDRATE AND NITROGEN METABOLISM IN *AGROSTIS*, *CYNODON* AND *POA* SPECIES

by

John E. Kaufmann

Supraoptimal temperatures inhibit growth of cool season turfgrasses. This investigation was designed to evaluate changes in levels of metabolites and rates of metabolism within certain intermediary pathways under high temperature growth stoppage conditions. Toronto creeping bentgrass (*Agrostis palustris* Huds.), and Merion Kentucky bluegrass (*Poa pratensis* L.), were used because both are widely used cultivars of cool season species that exhibit growth stoppage at supraoptimal temperatures. These species were compared to Tifgreen bermudagrass (*Cynodon dactylon* L.), a warm season species having a higher temperature optimum for growth. Controlled environment chambers were used to simulate optimal and supraoptimal conditions for growth in the range of 20 to 35 C and 32 to 35 C, respectively. All other environmental parameters were held constant.

Maximum dry matter production was obtained at 25 C for both cool season species and at 35 C for the warm season species. Increasing temperature decreased the nitrogen content in Kentucky bluegrass and bermudagrass leaves. No change in the level of nitrogen was found in creeping bentgrass due to temperature changes.

Uptake of glucose-^{14}C, glutamine-^{14}C, leucine-^{14}C, and acetyl-CoA-^{14}C, was increased in creeping bentgrass, reduced in Kentucky bluegrass, and not affected in bermudagrass by increasing the preconditioning growth temperatures from 25 to 35 C. The higher temperature caused increased incorporation of all radioactive precursors into protein in all three species.

Exposure of the tissue to light for 14 hours increased the carbohydrate content of both Kentucky bluegrass and creeping bentgrass, and increased the respiration of glucose-^{14}C in Kentucky bluegrass. At a 35 C preconditioning temperature the extremes in diurnal variation of the carbohydrate content were moderated, and the respiration of glucose-^{14}C was decreased in both Kentucky bluegrass and creeping bentgrass. Increasing incubation temperatures from 20 to 35 C, increased the respiration of glucose-^{14}C, and decreased the percent incorporated into protein-^{14}C.

As the incubation temperatures were increased above 26 C for Kentucky bluegrass previously grown at 26 C,

the content of glutamine and asparagine increased; aspartate decreased; and glutamate and gamma aminobutyrate were unchanged. When grown at 32 C, a significant decrease in the glutamine content occurred when the tissue was incubated at increasingly higher temperatures.

As incubation temperatures were increased above 26 C for Kentucky bluegrass grown at 26 C, the percent glutamine-^{14}C found in the tissue increased; the percent glutamate-^{14}C decreased; and the percent aspartate-^{14}C, asparagine-^{14}C, and $^{14}CO_2$ evolved was increased. As the incubation temperature was increased from 26 C to 38 C the percent glutamine-^{14}C was halved for Kentucky bluegrass grown at 32 C while the percent evolved as $^{14}CO_2$ was doubled.

When Kentucky bluegrass was placed under acute heat stress, glutamine accumulation occurred with little conversion to glutamate. However, when Kentucky bluegrass was under chronic heat stress, rapid conversion of glutamine to CO_2 occurred, resulting in depletion of the glutamine pool. It was suggested that loss of the glutamine pool resulted in reduced synthesis of certain growth sustaining proteins or other macromolecules that require glutamine as a precursor.

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INTRODUCTION

The effect of supraoptimal temperatures in inhibiting the growth of cool season grasses has been known since the 1930's. Early investigations suggested that carbohydrate levels were lowered with increasing temperature. Thus high temperature growth stoppage was attributed to a lack of carbohydrates for growth.

A revolution in turfgrass science in the 1950's resulted from: (a) development of improved cultivars of both warm and cool season species, (b) availability of fertilizers, pesticides, and fungicides formulated for turf use, and (c) development of turfgrass maintenance equipment. New cultivars of cool season species provided high quality turfs in the north while warm season cultivars were well adapted in the south. However, the transition zone lacked a high quality turfgrass due to summer dormancy in cool season turfgrasses. In an effort to overcome dormancy, fertility, irrigation, disease control and maintenance practices were adjusted. However, supraoptimal temperatures, an aspect of the environment which could not be controlled, were causing growth stoppage of the cool season turfgrasses.

Since that time, many investigations concerning the effect of temperature on levels of various carbohydrate and nitrogen fractions have been conducted. There is a widely accepted hypothesis that when turfgrasses are grown at optimal temperatures, net photosynthesis exceeds net respiration, resulting in carbohydrate accumulation. However, when turfgrasses are grown at supraoptimal temperatures net respiration overcomes net photosynthesis resulting in loss of available carbohydrates. If temperatures remain above the optimum for growth, carbohydrates are exhausted and growth stops.

A second hypothesis supported by more recent investigations proposes that the carbohydrate levels are inversely related to growth. Thus, as supraoptimal temperatures are approached, growth is reduced and carbohydrate levels increase.

Exhaustion of the glutamine pool has been shown to occur at supraoptimal temperatures (4), but the exact relationship between loss of glutamine and growth stoppage has not been determined.

The objectives of this investigation were to: (a) compare the effect of temperature on certain aspects of intermediary metabolism in three turfgrass species, (b) reevaluate the effect of temperature on carbohydrate

levels and metabolism, and (c) determine the effect of temperature on metabolism of the glutamine pool and relate it to growth stoppage.

LITERATURE REVIEW

Effects of Temperature on Growth

The effects of temperature on growth have been investigated extensively. Brown (7) concluded that soil temperature was more important than air temperature in reporting temperature effects on growth. 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.

Stuckey (37) reported that a soil temperature of 26.5 C accelerated maturation of colonial bentgrass (Agrostis tenuis Sibth.) roots. It was proposed that plant death at this temperature was due to early maturation and death of the root system.

Mitchell (23) found that 20 C was the optimum temperature for dry weight production of perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis alomerata L.), colonial bentgrass, and velvetgrass (Holcus lanatus L.). In contrast, the optimum for Dalligrass (Paspalum dilatatum Pois.) was near 30 C. A rapid decline in growth occurred above the optimum temperatures with growth of the cool-season grasses ceasing above 35 C.

Sullivan and Sprague (38) reported that root dry-matter production of perennial ryegrass was greatest at the

coolest temperature (15.6/10 C, day/night), and lowest at the warmest temperature regime (32.2/26.7 C). 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.

Harrison (12) reported that shoot growth of Kentucky bluegrass was higher at 26.7 C than at 15.6 C for the first 10-day cutting. The dry weight of the rhizomes at the end of the experiment decreased with increasing temperature while root weights were maximum at 15.6 C.

Schmidt and Blaser (30) noted maximum shoot growth of Cohansey creeping bentgrass after 45 days at 36 C. Reduced growth was found at 24 C and again at 12 C. Highest root production was at 12 C, and was decreased with increasing temperature. After switching temperatures for an additional 10 day period, root and shoot growth of bentgrass increased as temperatures were decreased from 36 C. However, when bentgrass maintained at 12 C was grown at higher temperatures, shoot growth increased while root growth decreased.

Watschke, Schmidt, and Blaser (42) observed growth of five Kentucky bluegrass cultivars at day/night temperature regimes of 18/10, 27/18, and 35/20 C. Shoot growth was greatest at the two lower temperatures and was reduced at the highest temperature. Maximum root production occurred at 27/18 C and minimum at 35/20 C. Those Kentucky bluegrass cultivars selected from a warm climate were better adapted to heat stress.

In a recent investigation by Watschke et al. (41), 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 the first week, but less than growth at 23/15 C on subsequent weekly harvests.

Younger and Nudge (47) reported that of three Kentucky bluegrass cultivars grown at day/night temperature regimes of 27/21, 27/16, 18/12, and 16/7 C, shoot growth was greatest at the warmer day temperatures and decreased with decreasing temperature. In Fylking and Newport, shoot growth was highest at 27/16 C, while maximum growth of Merion was found at 27/21 C. Merion produced significantly more dry matter than the other two cultivars.

McKell et al.(21) compared foliar growth of Coastal bermudagrass and Newport Kentucky bluegrass at four day/night temperature regimes ranging from 13/7 to 30/24 C. Shoot growth of Newport Kentucky bluegrass grown at 18/13 C increased with each sampling date. Shoot growth at the other three temperatures was similar on the first sampling date but did not increase on subsequent sampling dates. Bermudagrass shoot growth increased as temperatures increased.

The shoot growth rate of Toronto creeping bentgrass was measured by Duff (10) at five day/night temperature

regimes from 20/10 to 40/30 C. Shoot growth was maximum at 20/10 C and decreased with each increment of increased temperature. Martin (20) found that 25 C was the optimum for shoot growth of Merion Kentucky bluegrass. As temperature increased growth was reduced. Severe growth reductions were noted at a constant day/night temperature of 35 C.

The shoot growth optimum for cool season grasses such as creeping bentgrass and Kentucky bluegrass is in the range of 20 to 25 C. Warm season species such as bermudagrass and dallisgrass exhibit growth optimums of 30 to 35 C. Root growth optimums appear to be approximately 5 C less than shoot growth optimums.

Effects of Temperature on Carbohydrate Levels

The effect of many environmental factors on growth and carbohydrate content of leaf tissue has been investigated extensively. Factors which promote dry weight production generally cause a reduction in carbohydrate levels, or do not permit carbohydrate levels to be restored to high levels after a period of carbohydrate utilization.

Seasonal fluctuations in temperature affect the carbohydrate level in grasses. Brown (7) reported production and storage of carbohydrates in Kentucky bluegrass during the cool temperatures of spring. Loss of stored carbohydrates from roots and rhizomes occurred during the summer. However, carbohydrate storage again occurred in

the fall. It was not determined whether the summer reductions were a result of increased growth at optimal temperature or growth stoppage at supraoptimal temperatures.

Another investigation of seasonal effects by Zanoni et al. (48) showed that Merion Kentucky bluegrass had increasing carbohydrate levels from late spring to midsummer, a sharp drop in late summer, and an increase during the fall.

Harrison (12) attributed reduced growth of Kentucky bluegrass at higher temperatures to carbohydrate exhaustion. The data of Sullivan and Sprague (38) indicated that maximum carbohydrate accumulation occurred in perennial ryegrass at a day/night temperature of 21.1/15.6 C, with a rapid decrease in carbohydrates at temperatures above the optimum for growth. The highest percentage of carbohydrates in ryegrass was found in the subble, with reduced amounts in the leaves and roots.

The reserve carbohydrate exhaustion theory was advanced when Schmidt and Blaser (30) reported that 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 also decreased as temperature increased.

Youngner and Nudge (47) reported that high growth temperatures resulted in lower carbohydrate reserves in

Kentucky bluegrass. However, supraoptimal temperatures for growth were not included in this investigation since increasing temperature was found to increase growth. In a subsequent investigation in the same laboratory, McKell et al. (21) measured reduced fructosan levels at higher growth temperatures in Newport Kentucky bluegrass where growth was reduced. Increased growth of Coastal bermudagrass reflected a reduction of starch reserves at the same high temperature.

Brown and Blaser (8) found similar carbohydrate levels in orchardgrass grown at 35 C and at 24 C. They attributed the lack of reduced carbohydrate levels to moisture stress which overcame the effects of higher temperature.

Watschke, Schmidt, and Blazer (42) reported that carbohydrate levels in five Kentucky bluegrass cultivars decreased as the growth temperature was increased to a maximum day/night regime of 30/20 C. It was also concluded that cultivars with a high carbohydrate content best supported growth at the high temperatures.

Watschke, et al. (41) found that foliar carbohydrate level in ten Kentucky bluegrass cultivars decreased as the day/night growth temperature was increased from 23/15 to 35/25 C. Even though carbohydrate level was as high as 14.1% after four weeks at the 35/25 C temperature, the authors still contended that threshold carbohydrate levels were causing reduced dry matter production.

Supraoptimal temperatures for growth of cool season grasses have been shown to increase the carbohydrate level in both creeping bentgrass and Kentucky bluegrass. Duff (10) reported that both the 85% ethanol-soluble carbohydrate level and the water-soluble carbohydrate level increased in creeping bentgrass as day/night temperatures were increased from 20/10 C to 40/30 C.

The investigation by Martin (20) indicates that carbohydrate levels were inversely related to growth of Merion Kentucky bluegrass. Minimum carbohydrates levels were found at 25 C where growth was maximum. Maximum carbohydrate levels were found at 35 C where dry matter production was severely impeded.

Effects of Nitrogen Nutrition

Nitrogen is an essential element for the growth of grasses. When applied to turfgrasses grown under optimum conditions it increases dry matter production. However, the reduction in growth of Italian ryegrass (Lolium multiflorum Lam.) caused by above optimum temperatures was even greater at high nitrogen levels (36).

Pellett and Roberts (27) found that Kentucky bluegrass turfs grown at low nitrogen levels were more tolerant of high temperatures than when grown at high nitrogen levels. Harrison (12) found that removing leaf tissue from Kentucky bluegrass plants supplied with a nitrogen-free

nutrient solution, was less harmful during the hot summer months than was excessive defoliation of bluegrass plants which received a continuous supply of nitrogen. He also reported that after several defoliations, plants grown at 26.4 C and supplied with nitrogen produced no more top growth than nitrogen-free cultures.

Fertilization with nitrate has been shown superior to ammonium nitrogen in supporting greater shoot growth and rhizome development in Kentucky bluegrass (12), and has been shown to be effective over a wider range of soil pH's and temperatures (9). Stoin (36) indicated that at a 21/16 C day/night temperature high rates of nitrate were more effective in promoting growth of Lolium multiflorum Lam. than high rates of ammonium nitrogen. At a temperature regime of 32/26 C, nitrate was again more effective than ammonium nitrogen at both high and low levels of nutrition.

Sprague (31) also studied the effects of ammonium and nitrate nutrition on colonial bentgrass. Ammonium nitrogen reduced growth of shoots and roots when compared to nitrate. Temperature effects were not considered in this investigation.

Soluble carbohydrate levels in turfgrasses are inversely related to soil nitrogen levels. Orchardgrass and perennial ryegrass had higher carbohydrate levels at low soil nitrogen levels and both decreased with increased

nitrogen (1, 32). More recently, Lechtenberg et al. (18) reported that nitrogen fertilization reduced the fructosan and pentosan concentration in tall fescue (Festuca arundinacea Schreb.).

Studies on Kentucky bluegrass indicated increased shoot growth and decreased foliar carbohydrate content with the addition of nitrogen fertilizer (27). High nitrogen reduced the levels of acid-extractable carbohydrates found in Cohansey creeping bentgrass according to Schmidt and Blaser (30).

The effect of nitrogen nutrition on reserve carbohydrates in the leaves of creeping bentgrass and Kentucky bluegrass was investigated by Green and Beard (11). Oligosaccharides and fructosans decreased with added nitrogen while simple sugars were not significantly affected. Total carbohydrate content was generally higher at reduced nitrogen levels.

Weissman (44) reported that in the shoots of seedling wheat (Triticum aestivum), total nitrogen was higher following an ammonium treatment when compared to a nitrate treatment. This increase in total nitrogen was found to be in the nonprotein fraction. Further study indicated that amides were accumulated in the tissue when in ammonium culture. However, 12% more asparagine than glutamine was isolated after ammonium treatment and 30% more glutamine than asparagine was isolated after the nitrate treatment.

In a recent investigation, Watschke et al. (42) reported that high nitrogen levels stimulated shoot growth of five Kentucky bluegrass cultivars at 18/10 C day/night temperatures but reduced growth at 35/20 C. Nitrate uptake was not affected by nitrogen levels of the nutrient solution. Foliar levels of carbohydrate, nitrate and ammonia also did not vary with nitrogen levels.

Effects of Diurnal Variation

Carbohydrate levels exhibit large fluctuations due to diurnal variation. These fluctuations are a result of net photosynthesis or carbohydrate accumulation during the light period, and respiration or carbohydrate utilization during the dark period. Diurnal variation of other metabolite levels, such as the nitrogenous compounds, has been suggested by Youngberg (46) to be a result of a dilution effect from highly variable carbohydrate levels.

Dirunal variation of the water-soluble carbohydrate content has been observed in forage grasses. Waite and Boyd (40) report that sucrose levels increased in ryegrass from about 5% at 9 a.m. to 7% at 3 p.m. Seventy percent of the daily increase was lost between midnight and 3 a.m.

Holt and Hilst (15) state that water-soluble carbohydrates in tall fescue increased from 6% at 6 a.m. to 9% at 6 p.m., while water-soluable carbohydrates in Kentucky bluegrass increased from approximately 5% to 8% during the same period of time.

Lechtenberg et al. (18) reported that the average sugar content of tall fescue increased from about 8% at 6 a.m. to about 10% at 6 p.m. Almost 37% of the daily increase in sucrose was respired or translocated between 6 p.m. and midnight, and the remaining 63% after midnight.

The effect of diurnal variation on the content of both water-soluble and 85% ethanol-soluble carbohydrate of Merion Kentucky bluegrass leaves was investigated by Martin (20). The water-soluble carbohydrate content was approximately 4% at 7 a.m., 8% at noon, and 13% when the light period ended at 9 p.m. The levels dropped to about 10% at midnight and were near 4% when the dark period ended at 7 a.m. Strict controls on the time of sampling are necessary in measuring other environmental effects on carbohydrate levels.

Photosynthesis and Respiration

Photosynthesis rates are affected by several environmental factors including temperature, light intensity, light quality, and carbon dioxide. Respiration rates are affected by temperature, light and oxygen concentration. When other factors are held constant, photosynthesis and respiration increase with temperature to a maximum rate then decrease. It has been widely accepted that the maximum rate of photosynthesis occurs at a lower temperature than for respiration. Therefore, at some point

along the temperature curve respiration rates overcome photosynthetic rates and a net loss of carbon skeletons occur.

Temperature optimum of photosynthesis between temperate and tropical origin forage grasses was reported by Murata and Iyama (25). Apparent photosynthesis in Italian and perennial ryegrass was greatest at 5 to 15 C, and at 35 C for bermudagrass and bahiagrass (Paspalum notatum Flugge). Respiration increased with increasing temperature through 40 C for all species.

Moss et al. (24) showed respiration increased in corn (Zea mays L.) more rapidly than photosynthesis as temperature was increased to 43.9 C. However, the magnitude of photosynthesis was so much greater that net assimilation was greater at the higher temperature.

According to Miller (22) maximum net photosynthesis of Seaside creeping bentgrass occurred at 25 C and was from 35 to 40 C for common bermudagrass. At a temperature of 40 C, photosynthesis was 62.2% of maximum in the cool season species, and 97.7% of maximum in the warm season species. Schmidt and Blaser (30) reported 80% more CO_2 fixed in Cohansey creeping bentgrass grown at 24 C when compared to 12 C or 36 C.

Duff (10) found that photosynthesis of leaf sections of Toronto creeping bentgrass grown at both 20/10 and 40/30 was greater at a test temperature of 30 C than 20 C or 40 C. When grown at 40/30 C., the reduction of photosynthesis caused by increasing test temperature from 30 C to 40 C was much less compared to 20/10 C. Maximum respiration was found at 40 C for both temperatures. However, the rates of photosynthesis at 40 C far exceeded respiration rates.

Data from ten bluegrass cultivars investigated by Watschke et al. (41) indicated that day/night temperatures of 35/25 C did not cause the sum of the rates of dark respiration and photorespiration to exceed photosynthetic rates measured in a low oxygen atmosphere.

Martin (20) found that increasing test temperatures at 5 C increments from 20 C to 40 C increased both respiration and photosynthesis of Merion Kentucky bluegrass regardless of the temperature of the preconditioning period. When preconditioned for one week at 35 C, however, both photosynthesis and respiration were reduced compared to plants grown for one week at 20 C. Respiration did not exceed photosynthesis at any temperature.

Nitrogen Metabolism

Nitrogen exists in leaf tissue as proteins, amino acids, amides, free ammonia, free nitrate, and other nitrogenous compounds. While temperature changes do not greatly alter total nitrogen, significant changes in the levels of one or more of the nitrogen containing compounds could be responsible for growth stoppage of cool season turfgrasses.

Steward (33) has suggested that environmental factors may interact with metabolic processes at the point of contact between carbohydrate and nitrogen metabolism involving keto acids, amino acids, and amides. His studies with the mint plant (Mentha pipereta L.) indicated daylight, long days, and night temperatures promoted protein synthesis and glutamine accumulation in the leaves. In contrast, darkness, short days, and high night temperatures favored asparagine accumulation.

Beard and Daniel (4) studied the seasonal variation in the total, nonprotein, glutamine, asparagine and total amide nitrogen fractions of creeping bentgrass leaf tissue. Temperature was the major environmental factor affecting seasonal variations in these fractions. Total nitrogen increased with average daily soil temperatures through 24 C and then decreased at higher temperatures. Glutamine decreased sharply with increasing

temperatures, dropping to very low values at soil temperatures above 24 C. Asparagine was also reduced, but to a lesser extent.

In another investigation by Beard (3), total nitrogen increased in both creeping bentgrass and bermudagrass with increasing temperature. Nonprotein nitrogen increased with increasing temperature in bentgrass but decreased with increasing temperature in bermudagrass. The free ammonia level increased from low to optimum temperatures and decreased at higher temperatures. The amide level in bermudagrass decreased with increasing temperature, but not of the magnitude of decrease found for bentgrass.

Stoin (36) indicated that the protein nitrogen content of tall fescue and perennial ryegrass was relatively unaffected by growth temperature, but the soluble amino nitrogen content was increased with temperature. In an earlier investigation, Stoin (35) concluded that the soluble nitrogen content was higher when Kentucky bluegrass was grown at 35 C compared to 21 C. Aspartate and glutamate levels decreased, while asparagine increased. Glycine, valine, alanine, serine, threonine, isoleucine, and lysine increased with increasing temperature. Glutamine was reported to be highly variable with no definite trend.

Watschke et al. (42) reported that nitrate uptake by five Kentucky bluegrass cultivars was stimulated by increasing temperatures. However, even though growth was reduced at this temperature, the increased nitrate uptake did not result in increased levels of foliar nitrate or ammonium nitrogen.

Kaufmann et al. (16) reported that nitrate reductase activity of creeping bentgrass leaf tissue grown at 35 C was reduced when compared to 25 C. Nitrate reductase isolated from bermudagrass exhibited activity through 40 C.

Data from Schmidt and Blaser (30) indicated that total nitrogen in the stolons of creeping bentgrass increased with increasing temperature. In the leaf tissue the minimum total nitrogen was found at 24/18 C compared to 12/10 C and 36/30 C day/night temperatures.

Sullivan and Sprague (30) found that the 80% ethanol-soluble nitrogen fraction increased relative to total nitrogen in perennial ryegrass leaf tissue following clipping. The largest increase of this fraction occurred at the highest temperature treatment. Total nitrogen declined with time after clipping but the

decline was much less at the highest temperature treatment. The authors suggested the possibility of ammonium toxicity due to a rapid digestion of proteins at high temperatures.

In a review of the biochemical aspects of temperature effects Langridge (17) listed five possible causes for high temperature effects. Several of the possible causes involved reduced synthesis or accelerated breakdown of enzymes and/or amino acids and therefore, would involve protein or nitrogen metabolism.

Petinov and Molokovskii (28, 29) reported an accumulation of ammonia in several plant species subjected to temperatures above 45 C. High temperature treatment caused intensified proteolysis, ammonia accumulation, impaired amino acid synthesis, and abnormal amino acid metabolism. The abnormal amino acid metabolism included accumulation of large amounts of gamma aminobutyrate.

They were able to partially overcome heat injury by treating the plans with organic acids. These, they suggested, neutralized the ammonia and provided energy sources for the neutralizing reaction. Ultimately the excess ammonia was stored in amide pools and was available for resynthesis of proteins.

Steward and Margolis (34) noted that a manganese deficiency produced an accumulation of amides in tomato

plants. This deficiency was found not to interfere with nitrate reduction or the immediate conversion of nitrogen to an organic form, but rather to control the supply of carbon accepters for nitrogen via Krebs cycle. Thus existing amino acids were converted to amides.

The effects of light on the metabolism of glucose and glutamine in wheat leaves were investigated by Bidwell et al. (6). In the light, 68% of radioactive glucose was converted to sucrose, while in the dark only 33.2% was isolated as sucrose. Greater quantities of aspartate, glutamate, asparagine, glutamine, alanine, and carbon dioxide were formed in the dark.

When radioactive glutamine was fed in the light, 30.5% of the radioactivity remained as glutamine and 28.9% was isolated as glutamate. In the dark 48.4% remained as glutamine and 16.0% was isolated as glutamate. Greater quantaties of asparagine, aspartate and carbon dioxide were isolated after the dark treatment, but greater quantities of sugars and other amino acids were isolated after the light treatment. Gamma aminobutyrate was isolated from glutamine fed wheat leaves after both light and dark treatments.

Glutamate metabolism of wheat leaves was investigated by Naylor and Tolbert (26). Under anerobic conditions the major product was gamma aminobutyrate (GAB). However, when oxygen was supplied, glutamine and several organic acids were the major products. The large accumulation of

GAB at low oxygen conditions was a result of either increased decarboxylation of glutamate or reduced transamination of GAB to succinate semiadehyde.

Synge (39) isolated GAB from perennial ryegrass, and found it to be present in quantities as high as 6% of nonprotein nitrogen. This indicates that GAB is a normal metabolite in a turfgrass species and not merely a product of abnormal amino acid metabolism as suggested by the above authors (29, 26).

Glutamate carboxylase of wheat leaves was investigated by Weinberger and Clendenning (43). Greater quantities of the enzyme were isolated in the older leaves than in the young leaves. Beevers (5) reported that the enzyme may be heat inactivated at temperatures above 30 C.

In order to investigate the effect of heat stress on carbohydrate and nitrogen metabolism, it was necessary to review a wide range of literature. Final identification of the mechanism of growth stoppage of cool season turfgrasses at supraoptimal temperatures may well involve a complex interaction of these metabolic processes.

MATERIALS AND METHODS

Establishment Procedures

Mature sods of Toronto creeping bentgrass and Merion Kentucky bluegrass were obtained from the Michigan State University experimental turfgrass field laboratory. The bentgrass and bluegrass had been maintained at a cutting height of 0.6 cm and 3.8 cm, respectively. The Tifgreen bermudagrass, maintained at a 1.27 cm cutting height, was shipped from Florida (A. E. Dudek, Plantation Field Laboratory, Ft. Lauderdale) as mature sod pieces approximately 25 x 25 cm.

The sod was trimmed to a 2.0 cm soil depth and placed in greenhouse flats (36 x 25 x 10 cm) previously lined with plastic and filled to a 6 cm depth with a sandy loam soil mix. Drainage holes were punched in the plastic. All flats were placed in a 20 to 25 C greenhouse under an automatic irrigation system for two weeks prior to imposing the temperature treatments. New sod of creeping bentgrass and Kentucky bluegrass was used for each experiment. Cutting heights for bluegrass, bentgrass, and bermudagrass were maintained at 5.0 cm, 2.0 cm, and 2.5 cm, respectively, during the entire investigation. The cutting height of bermudagrass and creeping bentgrass was raised to provide greater leaf blade length for sectioning in the experiments involving radiocarbon uptake.

Growth Conditions

The large environmental growth chambers (1.4 x 2.5 m) used for the experiments were maintained at a light intensity of 24,000 lux. and at a day length of 14 hours with day/night temperatures held constant. Following the greenhouse establishment period, the flats were transferred to the chambers and watered daily and twice daily when under optimal and supraoptimal temperatures, respectively. The samples were saturated twice weekly with a Hoaglands (14) micro-nutrient solution having macro nutrients modified to a 4:1:2 ratio of N, P, and K, respectively. An occasional dusting with malathion was necessary for insect control.

One chamber was used throughout the experiment on aspects of intermediary metabolism and was initially adjusted to a soil temperature of 20 C. Six flats of the three species were transferred to the chamber and the temperature was monitored with a thermometer placed at a 5 cm soil depth. Following a two week acclimation period, the temperature was increased 5 C every two weeks through 35 C. Four replications were harvested twice weekly for analysis of growth and nitrogen content. Three replications of fresh leaf tissue were sampled for the radiocarbon study during the final three days of the 25 C and 35 C growth periods. Sampling occurred after two hours of light duration.

In the carbohydrate metabolism experiment, six flats of both Merion Kentucky bluegrass and Toronto

creeping bentgrass were placed in each of two growth chambers adjusted to a constant day/night temperature of 20 and 35 C. After three weeks, four replications were harvested for the nitrogen, protein, and carbohydrate analyses. Three replications of fresh leaf tissue were sampled for the radiocarbon study during the final three days of the experiment. The effect of light duration was achieved by sampling just as the light period began (0 hours) and again just prior to the dark period (14 hours of light). When the temperatures during incubation were adjusted to 20 and 35 C, samples were taken after two hours light duration.

One growth chamber was used in the glutamine metabolism experiment. Six flats of Merion Kentucky bluegrass were grown at 26 C for three weeks. The chamber was then adjusted to 32 C for another three week period. Three replications of fresh leaf tissue were sampled following two hours of light duration during the final three days of both growth periods.

Sample Preparation

For growth, nitrogen, protein, and carbohydrate measurements, the tissue samples were frozen immediately in dry ice and carried to the laboratory where they were freeze-dried, ground in a Wiley mill, and stored in air-tight bottles.

In the radiocarbon studies, fresh tissue was collected, placed in an airtight plastic bag containing a moistened paper towel, and chilled to 0-3 C. A special cutting apparatus having three parallel razor blades at a 0.5 cm spacing was used to cut sections of fresh leaf blade tissue which were quickly transferred to a flask containing an uptake buffer (to be described in Incubation Procedures). Sixty sections of bluegrass and bermudagrass, and 80 sections of bentgrass were cut for each flask. This represented approximately 60 mg fresh tissue per flask.

Radioisotope Materials

Glucose, glutamine and leucine were obtained as 50 uCi each of uniformly labeled carbon-14. Ten uCi of acetyl-CoA were labeled on the carbonyl carbon of the acetyl group. Each incubation flask received an aliquot containing 0.5 uCi (0.404 ug) glucose dissolved in 10 ul 85% ethanol, 0.5 uCi (0.334 ug) glutamine dissolved in 10 ul water, 0.5 uCi (0.268 ug) leucine dissolved in 10 ul of 0.005 N HCl, or 0.2 uCi (3.26 ug) acetyl-CoA dissolved in 10.0 ul water.

Incubation Procedures

Warburg flasks with sidearm were used for the radioactive uptake studies. Two ml of chilled 0.05 M phosphate buffer (pH 6.4) were placed in the bottom of each flask. The

leaf blade sections were placed immediately in the buffer solution.

Ten ul of the radioactive chemical were placed in the uptake buffer and mixed thoroughly. Each flask was immediately covered with a rubber septum fitted with a glass tube inserted just below the buffer surface. The flasks were then placed in a temperature controlled water-bath maintained at 30 C throughout all experiments except when the effect of temperature during incubation was being studied. An aluminum foil cover was placed over the water-bath to insure a dark respiration measurement. The sidearm of the flask was connected to a vacuum apparatus containing a CO_2 trap. The $^{14}CO_2$ evolved from the tissue was trapped and counted. Flow rates of air through the flask were maintained at 30 ml per minute.

In the intermediary metabolism and carbohydrate metabolism experiment, incubation was allowed to continue for a period of two hours, after which the leaf sections were rinsed in a Buchner funnel and blotted dry. Half of the sections were transferred to a preweighed sample wrapper and placed in a 80 C oven. The other half were placed in a 10 ml teflon grinder, frozen with dry ice and placed in a freezer. Glucose was the only radioactive chemical used in the carbohydrate metabolism experiment.

In the glutamine metabolism experiment, glutamine was the only radioactive chemical used. All leaf sections were frozen immediately after a one hour incubation period.

Total ^{14}C-uptake Determination

The leaf sections that had been placed in the oven, were dried for 24 hours and weighed. The weight of the sections was doubled and used as a dry weight measurement for each sample. The sample was then combusted in an oxygen atmosphere and 20 ml of a mixture of ethanolamine and ethanol (1:2) was added to absorb the $^{14}CO_2$. A 2 ml aliquot was removed and placed in a scintillation vial with 10 ml scintillation solution for counting. Total uptake was expressed as dpm/mg dry weight/hour, and was determined by totaling the dpm of $^{14}CO_2$ and combustion.

In the glutamine metabolism experiment, the ^{14}C-compounds were extracted in one ml of cold methanol-water (50:50). The extract was filtered through a previously weighed Whatman No. 29 black sample wrapper. The cell wall debris was oven dried, weighed and combusted to $^{14}CO_2$ to determine the radioactivity. The filtrate was stored in a cold chamber.

The weight of the cell wall debris was used to adjust the data to a per mg dry weight basis. The dpm of combustion, of CO_2 evolved, and of the extract was summed to provide a measurement of total uptake (dpm/mg dry wt/hr).

Protein-^{14}C Analysis Procedure

The leaf sections placed in the freezer were ground in the teflon grinder with a phosphate buffer described

by Wilkinson and Beard (45). Grinding was complete in two minutes without heating and the sample was poured into a centrifuge tube using an additional 2 ml of buffer to rinse the grinder. The protein extract was separated at 15,000 x g for 20 minutes in a refrigerated centrifuge. The supernatant was decanted into a test tube and placed in an ice bath. A 0.6 ml aliquet was transferred to another test tube and 2.0 ml of 10% TCA was added. Protein precipitation was allowed to occur for a minimum of 10 minutes. The samples were removed from the ice bath and filtered through a 0.45 um millipore filter. This filter was placed in a scintillation vial and was completely dissolved by the 10 ml scintillation solution.

The scintillation solution for all samples contained: 0.10 g POPOP, 5.0 g PPO, 380 ml p-dioxane, 380 ml toluene, and 240 ml absolute ethanol. Counting efficiency was always in the range of 45 to 55 percent. All data from the radioisotope investigations were reported as total uptake of the radioactive chemical, percent of total uptake evolved as $^{14}CO_2$ and percent of total uptake incorporated into protein ^{14}C.

Thin Layer Chromatography Techniques

Two dimensional thin layer chromatography (TLC) was used in the separation of amino acids in the extract. A 0.5 mm layer of cellulose was spread on a 20 x 20 cm glass plate and allowed to dry for a period of 24 hours.

A 0.1 ml aliquot of the extract was spotted in the lower lefthand corner of the plate and was allowed to dry thoroughly before being chromatographed. The solvent system for the first direction was chloroform-methanol-17 percent ammonia (40:40:20). The second direction was phenol-water (75:25).

To insure accurate identification of the amino acids and radioactive spots, a mixture of Silica Gel G and cellulose (50:50) was substituted as the thin layer, and the solvent system for the first direction was replaced by butanol-acetic acid-water (60:20:20). It was determined that the radioactivity was being emitted from the amino acids. Twenty-one amino acid standards were chromatographed for the determination of Rf values.

Following development of the chromatogram, the plate was allowed to dry for 15 minutes, placed in a spray chamber and coated with a layer of Ninspray (ninhydrin) and transferred to an 80 C chamber for four minutes. After the oven treatment, the plate was allowed to cool two hours as the spots developed. The average density of each individual spot was recorded with a Photovolt densitometer. Through the use of chromatographed standards of known concentrations of amino acids, an estimate of the quantity of amino acids isolated from the extract was determined, and reported as ug amino acid/mg dry weight.

Following the densitometer measurements, the spots were scraped from the plate and placed in liquid

scintillation vials for counting. The radioactivity isolated from each amino acid was recorded as percent of total dpm. No areas of the TLC plate, with the exception of the origin and the five amino acid spots, were found to contain detectable radioactivity.

Dry Weight Production

The weight of the freeze-dried tissue was totaled for each temperature period and reported as mg dry weight/sq decim/wk.

Nitrogen Analysis

A modified micro Kjeldahl technique (2) was used in determining the total nitrogen content of the freeze-dried tissue. A 50 mg sample was used for bentgrass and bluegrass and a 75 mg sample was used for bermudagrass. Data were reported as percent nitrogen on a dry weight basis.

Protein Analysis

Fifty mg of the dry weight sample were placed in a Virtis blender with 50 ml of 0.2 M phosphate buffer (pH 7.0) containing 10 mM Naethylenediamine tetraacetic acid. The extract was centrifuged at 15,000 xg for 20 minutes and the supernatant decanted for analysis. The extract was kept cold (0 to 3 C) throughout the analysis. One half ml extract was assayed for protein using the Lowry protein test (19). Optical density was determined

with a Perkin-Elmer spectrophotometer adjusted at 660 mu and compared to a series of standards prepared with bovine serum albumen. Data were reported as percent protein on a dry weight basis.

Carbohydrate Analysis

A 50 mg sample was placed in a large test tube with 10 ml of 85% ethanol. The tube was stoppered and placed on an automatic shaker for one hour. Approximately 1 g activated charcoal was added to remove the chlorophyll, and the extract was filtered in a Buchner funnel through Whatman No. 42 filter paper. The ethanol was removed in a flash evaporator. The filtrate was transferred to a 100 ml volumetric flask and diluted to volume. One ml of extract was used for analysis. Analysis was based on the procedure described by Martin (20) utilizing the color reaction of the anthrone reagent with the carbohydrates contained in the sample. Optical density was determined with a spectrophotometer adjusted at 620 mu. A standard curve was provided by analyzing a series of known concentrations of glucose.

Statistical Analysis

Statistical significance of all data was determined with Duncan's multiple range test after obtaining an analysis of variance with a significant F test.

RESULTS

Intermediary Metabolism Experiment

The influence of temperature on the dry matter production and the nitrogen content of the leaves is shown on Table 1. Maximum dry matter production occurred

TABLE 1.--The effect of temperature on the growth and nitrogen content of three turfgrass species.

Species	Temperature C	Growth (mg dry wt/sq decim/wk)	Nitrogen (% dry wt)
Kentucky bluegrass	20	370 e	4.41 h
	25	510 g	4.26 g
	30	437 f	3.98 ef
	35	103 a	3.52 d
Creeping bentgrass	20	226 c	4.21 g
	25	272 d	3.77 e
	30	252 cd	4.36 g
	35	150 b	4.16 fg
Bermuda-grass	20	83 a	3.00 c
	25	184 b	2.89 c
	30	391 e	2.67 b
	35	503 h	2.38 a

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test)

at the 25 C growth temperature for Kentucky bluegrass and at 25 C to 30 C for creeping bentgrass. Significant reductions of dry matter were found as the growth temperature

was increased to 35 C. Maximum dry matter production occurred at 35 C for bermudagrass, while statistically significant reductions were noted at lower temperatures.

The nitrogen content decreased with increasing temperatures in bluegrass and bermudagrass. In creeping bentgrass the highest nitrogen content was found at 30 C and the lowest at 25 C. These are the temperatures where the growth rate was highest. The effect of temperature on the nitrogen content was inversely related to the effect of temperature on growth in bermudagrass and directly related in Kentucky bluegrass.

Uptake studies of four radiocarbon materials were conducted at maximum (25 C) and minimum (35 C) growth temperatures for the two cool season species. Supra-optimal growth temperatures reduced the uptake of glucose-^{14}C in Kentucky bluegrass, increased uptake in creeping bentgrass, and did not affect uptake in bermudagrass (Table 2). Increased temperature reduced the percent $^{14}CO_2$ evolved, and increased the percent protein-^{14}C synthesized in all three species.

When glutamine-^{14}C was used as the source, total uptake was increased with temperature in creeping bentgrass (Table 3). Uptake in Kentucky bluegrass and bermudagrass were not affected. Increased temperature during growth increased the percent $^{14}CO_2$ evolved in Kentucky bluegrass. In creeping bentgrass, the percent $^{14}CO_2$ evolved was reduced

TABLE 2.--The effect of temperature during growth on the ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from glucose-^{14}C by leaf sections of three turfgrass species.

Species	Growth temperature (C)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Kentucky bluegrass	25	6394 b	24.4 b	1.14 b
	35	2681 a	19.4 a	1.30 c
Creeping bentgrass	25	6939 b	30.1 c	1.91 d
	35	10508 c	25.8 b	3.54 e
Bermuda-grass	25	1701 a	40.4 d	0.68 a
	35	2451 a	30.5 c	1.88 d

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test)

TABLE 3.--The effect of temperature during growth on the ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from glutamine-^{14}C by leaf sections of three turfgrass species.

Species	Growth temperature C	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Kentucky bluegrass	25	2032 a	37.4 a	0.74 a
	35	1859 a	45.1 b	1.84 c
Creeping bentgrass	25	3381 b	58.8 c	0.78 a
	35	9249 c	50.6 b	2.15 c
Bermuda-grass	25	1572 a	64.7 d	0.40 a
	35	2035 a	69.3 d	1.41 b

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test)

by supraoptimal temperatures while bermudagrass was unaffected. Synthesis of protein-^{14}C was increased significantly with increasing temperatures in all three species.

Leucine is an amino acid which enters into relatively few metabolic reactions. The total uptake of leucine-^{14}C was in the same general range as that found for glutamine, but the percent evolved as $^{14}CO_2$ was reduced (Table 4). Supraoptimal temperatures

TABLE 4.--The effect of temperature during growth on the ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from leucine-^{14}C by leaf sections of three turfgrass species.

Species	Growth temperature (C)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Kentucky bluegrass	25	3438 b	7.9 b	8.40 c
	35	2616 b	4.9 a	11.41 d
Creeping bentgrass	25	2972 b	16.5 c	6.71 b
	35	8710 c	7.0 b	15.71 e
Bermuda-grass	25	1234 a	20.6 d	1.34 a
	35	1230 a	20.9 d	9.43 c

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test)

during growth reduced the percent $^{14}CO_2$ evolved from the cool season species but had no effect on the warm season species. Synthesis of protein-^{14}C from leucine was found in very high percentages compared to that from other

radioactive substrates. Increasing temperatures during growth from 25 to 35 C resulted in statistically significant increases in synthesis of protein-^{14}C in all three species.

Total uptake of the relatively large acetyl-CoA molecule was approximately one-tenth of the other radiocarbon sybstrates (Table 5). Differences in uptake due to species and temperature, however, were similar to those found for glucose and glutamine. Supraoptimal temperatures increased the percent evolved as $^{14}CO_2$ from Kentucky bluegrass and creeping bentgrass, but reduced the percent evolved from bermudagrass. Temperature increased the percent protein-^{14}C synthesized in all three species.

Carbohydrate Metabolism Experiment

The effect of light duration on the percent ethanol-soluble carbohydrates was determined to see if there was any effect on the uptake of radioactive glucose (Table 6). Carbohydrate levels were greater after 14 hours light when Kentucky bluegrass and creeping bentgrass were grown at 20 C. However, when grown at 35 C, the diurnal variation in carbohydrate content was moderated. Therefore, if carbohydrates were measured early in the morning, a statistically significant increase was found with increasing temperature, but if carbohydrates were measured in the evening, a slight decrease was found for Kentucky bluegrass.

TABLE 5.--The effect of temperature during growth on the ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from acetyl-CoA-^{14}C by leaf sections of three turfgrass species.

Species	Growth temperature (C)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Kentucky bluegrass	25	261 a	18.7 a	2.55 a
	35	156 a	21.7 b	5.57 c
Creeping bentgrass	25	495 b	22.4 b	2.56 a
	35	855 c	36.2 d	4.09 b
Bermuda-grass	25	127 a	37.1 d	2.97 a
	35	131 a	27.7 c	6.21 c

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test).

TABLE 6.--The effect of temperature and light duration on the nitrogen, protein and carbohydrate content of two cool season turfgrass species.

Species	Growth temperature (C)	Light duration (hrs)	Content (% dry wt)		
			Nitrogen	Protein	Carbohydrate
Kentucky bluegrass	20	0	5.31 e	14.2 d	4.0 a
		14	4.95 d	13.8 d	17.1 f
	35	0	4.54 bc	12.9 bc	11.8 d
		14	4.27 ab	12.5 b	14.6 e
Creeping bentgrass	20	0	4.50 bc	10.5 a	3.1 a
		14	4.07 a	9.8 a	9.8 c
	35	0	4.92 d	13.4 cd	6.1 b
		14	4.66 cd	12.5 b	9.1 c

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test).

The nitrogen content decreased in Kentucky bluegrass and increased in creeping bentgrass with increasing temperatures. This response was similar to that reported in Table 1.

Increasing growth temperatures reduced the protein content of leaf tissue of Kentucky bluegrass and increased the protein content in creeping bentgrass. These changes, however, were not of the magnitude observed for nitrogen with the same temperatures. A fraction of the nitrogen content other than protein was being altered by supraoptimal temperatures. After 14 hours of light, the nitrogen content of both species grown at 20 C was reduced, but no effect was found when grown at 35 C. The protein content was not affected by light except in creeping bentgrass grown at 35 C where the content was reduced.

Table 7 indicates the effect of temperature and light duration on the uptake, percent $^{14}CO_2$ evolved and percent protein-^{14}C synthesized from radioactive glucose by leaf sections of Kentucky bluegrass and creeping bentgrass. Light was found to significantly reduce the amount of glucose-^{14}C uptake at 20 C and 35 C for creeping bentgrass. Light did not affect uptake in Kentucky bluegrass. Supraoptimal temperatures reduced uptake in Kentucky bluegrass and increased uptake in creeping bentgrass regardless of light duration.

TABLE 7.--The effect of temperature and light duration on the ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from glucose-^{14}C by leaf sections of two cool season turfgrass species.

Species	Growth temperature (C)	Light duration (hrs)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Kentucky bluegrass	20	0	8166 b	21.7 b	1.41 a
		14	5942 b	24.5 cd	1.37 a
	35	0	1996 a	18.7 a	2.40 d
		14	2780 a	25.4 d	1.77 bc
Creeping bentgrass	20	0	10197 c	26.2 d	1.55 ab
		14	7342 b	22.5 bc	1.31 a
	35	0	19916 d	23.0 bc	1.93 c
		14	10006 c	22.4 bc	2.52 d

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test).

TABLE 8.--The effect of temperature during growth and incubation on the ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from glucose-^{14}C by leaf sections of two cool season turfgrass species.

Species	Temperature Growth (C)	Temperature Incubation (C)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Kentucky bluegrass	20	20	2545 b	13.3 c	1.80 bc
		35	4004 c	18.5 e	1.10 a
	35	20	697 a	6.9 a	2.54 d
		35	2572 b	11.0 b	2.00 bcd
Creeping bentgrass	20	20	3817 c	12.7 c	2.47 cd
		35	5035 d	16.7 d	1.46 ab
	35	20	3342 c	10.8 b	3.75 e
		35	7617 e	17.3 de	2.66 d

Values with the same letter within vertical columns are not significant at the 5% level (Duncan's Multiple Range Test).

After 14 hours of light the $^{14}CO_2$ evolved in Kentucky bluegrass was increased. The protein-^{14}C synthesized in Kentucky bluegrass was decreased by maximum light duration at 35 C. In creeping bentgrass the $^{14}CO_2$ evolved was decreased at 20 C and the protein-^{14}C increased at 35 C with increasing light duration. The higher temperature reduced the percent $^{14}CO_2$ evolved for both species at 0 hours light duration. No effects of temperature on $^{14}CO_2$ were found after 14 hours light duration. Supraoptimal temperatures stimulated protein-^{14}C synthesis in both species at both 0 and 14 hours of light.

The effect of temperature during growth and incubation on the uptake, percent $^{14}CO_2$ evolved, and protein-^{14}C synthesized from glucose-^{14}C is found in Table 8. The higher temperature for growth significantly reduced the amount of uptake in Kentucky bluegrass, while the higher temperature during incubation increased uptake. The higher temperature for incubation increased uptake in leaf sections of creeping bentgrass. The higher temperature for growth increased uptake at 35 C incubation and did not affect uptake at 20 C.

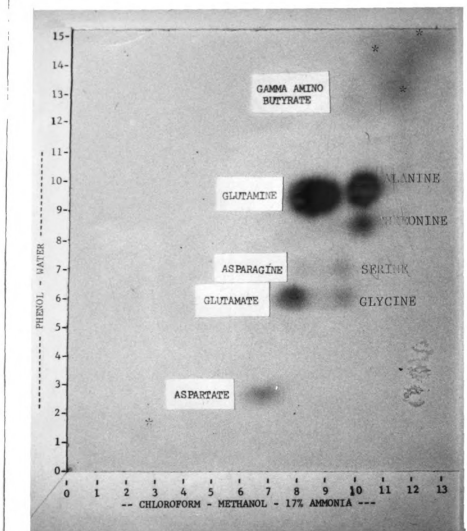
The percent $^{14}CO_2$ evolved was always increased at the high temperature during incubation which is due to normal enzyme response to temperature. Supraoptimal temperature during growth increased respiration in Kentucky

bluegrass at both temperatures during incubations and in creeping bentgrass incubated at 20 C. Supraoptimal temperatures for growth stimulated protein-^{14}C synthesis regardless of the temperature during incubation. It should be noted however, that more rapid synthesis of protein-^{14}C occurred when the temperature during incubation was 20 C rather than 35 C.

Glutamine Metabolism Experiment

Thirteen spots were isolated by the solvent systems of which nine spots could be accurately identified through the use of chromatographed standards (Figure 1). Five of the spots that were identified contained ^{14}C-labeled amino acids. They were identified as glutamine (GLM), glutamate (GLA), gamma aminobutyrate (GAB), aspartate (ASA), and asparagine (ASN). Threonine, alanine, glycine, and serine were also identified, but contained no detectable radioactivity.

Of the five amino acids which contained radioactivity, glutamine was present in the largest quantity (Table 9). As temperature during incubation was increased above 26 C, a significant accumulation of GLM occurred when grown at 26 C. At 32 C, increasing temperatures during incubation resulted in a significant reduction of



*Spots could not be positively identified.

Figure 1.--Position of thirteen amino acids isolated on a 0.5 mm layer of cellulose by thin layer chromatography.

TABLE 9.--The effect of temperature during growth and incubation on the content of five amino acids isolated from leaf sections of Merion Kentucky bluegrass.

Temperature (C)		ug/mg dry wt				
Growth	Incubation	Glutamine	Glutamate	Gamma Amino Butyrate	Aspartate	Asparagine
26	26	12.23 b	6.05 a	0.90 a	7.45 c	1.18 a
26	32	25.95 d	6.33 a	1.10 a	4.75 b	4.08 b
26	38	24.25 d	5.90 a	1.05 a	3.55 ab	4.30 b
32	26	20.70 c	6.03 a	-- *	6.35 c	4.20 b
32	32	10.13 b	6.28 a	1.08 a	4.38 b	3.55 b
32	38	4.05 a	5.88 a	0.93 a	2.35 a	-- *

Values with the same letter within vertical columns are not significant at the 1% level (Duncan's Multiple Range Test).

*Absent values are due to insufficient quantities of amino acid for detection by the ninhydrin spray.

the GLM content of the leaf tissue. No differences in the quantity of glutamate were detected.

The gamma aminobutyrate content of the tissue was small but detectable. The values in Table 9 are not significantly different from each other. The ninhydrin spray was unable to detect less than 0.90 ug of GAB. Therefore, the absent value could range anywhere from 0 to 0.9 ug GAB.

Significant reductions in the aspartate content occurred as temperatures during incubation increased above 26 C at both temperatures during growth. When grown at 32 C, another reduction occurred at 38 C. No significant differences were found due to temperatures during growth.

The asparagine content of the leaf sections grown at 26 C increased significantly when the temperature during incubation increased above 26 C. When grown at 32 C however, the highest ASN content was found at the 26 C and 32 C temperatures during incubation. The absent value was due to the fact that the ninhydrin spray could not detect less than 1.10 ug ASN.

The effect of temperature on uptake of glutamine-^{14}C is included in Table 10. The higher growth temperature and increasing temperatures during incubation above 26 C reduced total uptake.

TABLE 10.--The effect of temperature during growth and incubation on the ^{14}C-uptake and ^{14}C-metabolites synthesized from glutamine-^{14}C by leaf sections of Merion Kentucky bluegrass.

Temperature (C)		(dpm/mg dry wt/hr)	Percent of Total Uptake					
Growth	Incubation	Total Uptake	Glutamine	Glutamate	Carbon Dioxide	Gamma Amino Butyrate	Aspartate	Asparagine
26	26	3267 d	23.5 b	37.3 cd	10.5 a	3.5 b	4.8 a	1.0 a
26	32	2860 cd	32.1 c	27.6 b	13.9 b	3.6 b	5.6 b	1.6 b
26	38	2641 bc	30.4 c	15.9 a	13.8 b	3.3 b	5.9 b	2.1 c
32	26	2389 b	24.7 b	36.8 c	10.6 a	4.1 c	5.5 b	- *
32	32	1957 a	14.6 a	40.2 d	17.5 c	2.3 a	4.9 a	1.3 ab
32	38	1901 a	12.6 a	34.7 c	21.8 d	3.0 b	4.7 a	2.3 c

Values with the same letter within vertical columns are not significant at the 1% level (Duncan's Multiple Range Test).

*Absent value is due to insufficient resolution of dpm from adjacent spots.

The percent of total uptake that remained as GLM in the tissue at the end of the incubation period is shown in Table 10. No effects of temperature during growth were found during a 26 C incubation. However, as temperatures during incubation increased above 26 C, Kentucky bluegrass grown at 26 C increased in the precent of GLM-^{14}C while that grown at 32 C decreased significantly.

The first product of GLM catabolism is GLA. When the leaf tissue is grown at 32 C, the largest percent GLA is noted at an incubation of 32 C. This percentage and the other percentages of GLA recorded at the 32 C temperature during growth do not differ significantly from the percent GLA found when grown and incubated at 26 C. Increasing temperatures during incubation significantly reduced the percent GLA isolated from the leaf sections when grown at 26 C.

The percent $^{14}CO_2$ evolved was higher when incubated above 26 C. A significantly higher rate of $^{14}CO_2$ was evolved at both 32 and 38 C when the leaf tissue was grown at 32 C rather than 26 C (Table 10). The marked increase in $^{14}CO_2$ evolved at a 38 C incubation found when growth occurred at 32 C but not at 26 C, would indicate an adaptive mechanism of the turfgrass in respiration of glutamine.

Gamma aminobutyrate contained relatively small percentages of the radioactive carbon (Table 10). Tempera-

tures of incubation did not significantly affect GAB-^{14}C when grown at 26 C. When grown at 32 C, a significantly higher percentage of GAB-^{14}C is noted at a 26 C incubation, and significantly lower at 32 C. The value for 38 C was not different from all three values at the 26 C temperature for growth.

Radioactive aspartate was isolated in slightly higher percentages than was GAB. Increases were noted for incubations above 26 C for growth at 26 C, and decreases were noted for growth at 32 C. This incubation temperature response parallels that noted for the percentage of GLM-^{14}C.

Asparagine synthesized from GLM-^{14}C was detected in very small percentages. A value could not be recorded for growth at 32 C and incubation at 26 C because the counts were so low that it was impossible to distinguish those of ASN from both background and stray GLA counts on the TLC plate (see Figure 1.) The percentage of ASN-^{14}C increased significantly at higher temperatures of incubation when grown at both 26 C and 32 C.

The total percentage of the six recorded metabolites in Table 10 does not equal 100%. Part of the discrepancy is due to the dpm of combustion, and part is due to dpm isolated in the extract that were not recovered on the TLC plates. Sugars, organic acids, and impurities probably account for the undetected radioactivity.

DISCUSSION

The warm season species, bermudagrass, responded differently to high (35 C) growth temperatures than both cool season species. However, Kentucky bluegrass and creeping bentgrass do not appear to have the same metabolic charachteristics at 35 C.

As growth temperatures increased from 20 C to 35 C, Kentucky bluegrass leaves became thicker, tougher and less succulent, while the leaf blades of creeping bentgrass were more tender and succulent. No morphological changes in bermudagrass leaves were observed.

Differences between species due to supraoptimal temperature were noted in the uptake of the radiochemicals. Uptake decreased in Kentucky bluegrass, decreased in creeping bentgrass, and was not affected in bermudagrass. The major portion of radiochemical uptake would probably be through the wound when excised leaf sections are floating in a buffer solution. Direct absorbance into the cells along the edge of the wound and uptake into internal cells through the severed xylem vessels would probably account for the total uptake measured. It is conceivable that increasing temperatures could either increase or decrease the amount of radiochemical harbored

in these vessels, but it would seem more likely that cell membrane permeability is affected. If that is true, then supraoptimal growth temperatures precondition Kentucky bluegrass for reduced membrane permeability, and increased permeability in creeping bentgrass.

Carbohydrates are generally considered to be the primary source of carbon skeletons for growth and respiration. However, under the conditions of the intermediary metabolism experiment, glucose uptake exceeded that of glutamine. Glutamine was respired at higher percentages in all species regardless of growth temperature. If this occurred naturally in leaf tissue, turfgrasses would be devoid of a glutamine pool which is not the case at optimum temperatures for growth (4). It is therefore likely that glutamine-^{14}C fed to an excised leaf section is more available for respiration than the existing glutamine pool at optimum temperatures. At supraoptimal temperatures, for growth of Kentucky bluegrass, significant increases in respiration of glutamine and decreases in respiration of glucose indicate evidence of an adaptive mechanism which was found only in bluegrass under the conditions of the experiment.

In Table 8, supraoptimal temperatures for growth increased protein-^{14}C synthesis while increasing incubation temperatures reduced protein-^{14}C synthesis. However, it

must be noted that no difference in incorporation of ^{14}C into protein was found between leaves grown and incubated at 20 C and those grown and incubated at 35 C. Thus supra-optimal temperatures do not appear to affect the synthesis of protein-^{14}C from three radioactive metabolites.

The increase in protein-^{14}C synthesis with increasing growth temperatures in Tables 2-5, may represent an increased synthesis and perhaps a rapid turnover rate of a few specific high temperature induced enzymes, and therefore, may not represent synthesis of proteins necessary for growth.

Protein-^{14}C synthesis from glutamine exhibited a 2.5 to 3 fold increase at the high temperature (Table 3). This increase was higher than that found for the other radiochemical substrates. It is possible that at supra-optimal temperatures for growth, proteins which require glutamine as a precursor are not being synthesized.

Martin (20) states that the best time to determine the effect of temperature on available carbohydrates for growth and respiration is at the beginning of the photo-synthetic period because this is the time that potential carbohydrate deficits would occur. Therefore, the effect of growth temperature on the uptake and metabolism of glucose-^{14}C should be monitored during these low carbo-hydrate levels in order to determine whether or not supraoptimal growth temperatures inhibit the metabolism of monosaccharides.

For example, Merion Kentucky bluegrass grown at 20 C is able to metabolize and respire carbohydrate levels down to 4 percent (Table 6) during the dark period. Then glucose-^{14}C was added to excised leaf sections and 21.7 percent of total uptake was respired as $^{14}CO_2$ (Table 7). When grown at 35 C, respiration in Kentucky bluegrass reduced the carbohydrate level to 11.8 percent during the dark period and subsequent incubation with glucose-^{14}C indicated the leaf tissue only respired $^{14}CO_2$ at 18.7 percent of total uptake. Supraoptimal temperatures have not only impaired leaf growth and increased carbohydrate levels, but also reduced the ability of the turfgrass plant to respire available monosaccharides.

If glucose-^{14}C metabolism was allowed to proceed after 14 hours of light, growth temperature had no effect on the percent $^{14}CO_2$ evolved. This would indicate that carbohydrate reserves were equally available and equally metabolized at both temperatures for growth after 14 hours of light.

The increased respiration of Merion Kentucky bluegrass at higher temperatures of incubation reported by Martin (20) was confirmed by the increased respiration of glucose-^{14}C as temperatures of incubation were increased (Table 8). Similar responses of reduced

respiration resulting from a preconditioning period at supraoptimal temperatures for growth were found.

Supraoptimal temperatures do not cause respiration to exceed photosynthesis (10, 20, 24, 41) and subsequent carbohydrate exhaustion. In fact, the opposite seems to be true.

The existence of the C4 pathway reported by Hatch and Slack (13) may appear to support the carbohydrate exhaustion theory since photosynthetic efficient plants generally include warm season turfgrass species while the C4 or inefficient plants include the cool season species.

It must be recognized that the C4 pathway is only more efficient in fixation of CO_2 to C4 dicarboxylic acids. Before carbohydrates can be synthesized, the path of carbon must proceed along the C4 pathway as in photosynthetically inefficient turfgrasses. Therefore, since interorganelle transport of four carbon organic acids has been proposed, it is very probable that the organic acids, some of which are Krebs cycle intermediates, are being utilized directly for respiration.

The photosynthetically efficient bermudagrass was found to respire glucose-^{14}C at a decreased percentage at 35 C (Table 2). This indicates that the higher photosynthetic efficiency of the C4 turfgrass plant does not result in increased carbohydrates available for respiration at supraoptimal temperatures.

The cool season turfgrasses that do not have this supply of 4-carbon dicarboxylic acids, utilize the glutamine pool as observed by Beard (4). A high temperature adaptive mechanism must be involved in determining the preference of the glutamine pool to the carbohydrate pool for respiration.

Glutamine is a relatively important metabolite in higher plants. It is known to exist in large quantities as storage for reduced nitrogen in the leaf tissue of turfgrasses, and is thought to be the primary transport molecule of reduced nitrogen throughout the plant. The results of this investigation support the presence of a glutamine pool in Merion Kentucky bluegrass when grown at optimal temperature and that exhaustion of this pool can occur at supraoptimal temperatures.

The glutamine pool can be metabolized in two ways. First, the ammonium group can be removed by deamination or in any one of numerous transamination reactions. Second, glutamine can be utilized for synthesis of proteins and other macromolecules.

Supraoptimal growth temperatures preconditioned a demand for protein-^{14}C synthesis in Kentucky bluegrass not only from glutamine-^{14}C, but also from three other radiocarbon substrates (Tables 2-5). In Table 6, the protein content was found to decrease with increasing

temperatures, and in Table 8, incubation at 35 C decreased protein-^{14}C synthesis from glucose. Therefore, depletion of a large glutamine pool could not be attributed to increases in protein synthesis. More likely the glutamine pool depletion is causing reduced rates of protein synthesis.

The known pathway of GLM metabolism is shown in Figure 2. GLA is the first product of GLM metabolism by a deamination reaction. GAB is the product of a direct decarboxylation of GLA while ASA and ASN are products of Krebs cycle decarboxylations.

GAB, ASA, and ASN were found to contain small percentages of radioactivity which were relatively unaffected by changes in temperature when compared to GLM, GLA, and CO_2 (Table 10). The combined percentages of GAB, ASA, and ASN do not equal that found for CO_2 especially at supraoptimal temperatures. This indicates the decarboxylation reactions were not driven by a need to fill a 4-carbon amino acid pool, but rather to satisfy an energy need through increased respiration.

Through the extraction of GAB and isolation of GAB-^{14}C, evidence exists that the alternate pathway to succinate outlined in Figure 2 is operative. However, other than the slight accumulation of GAB-^{14}C at 32 C

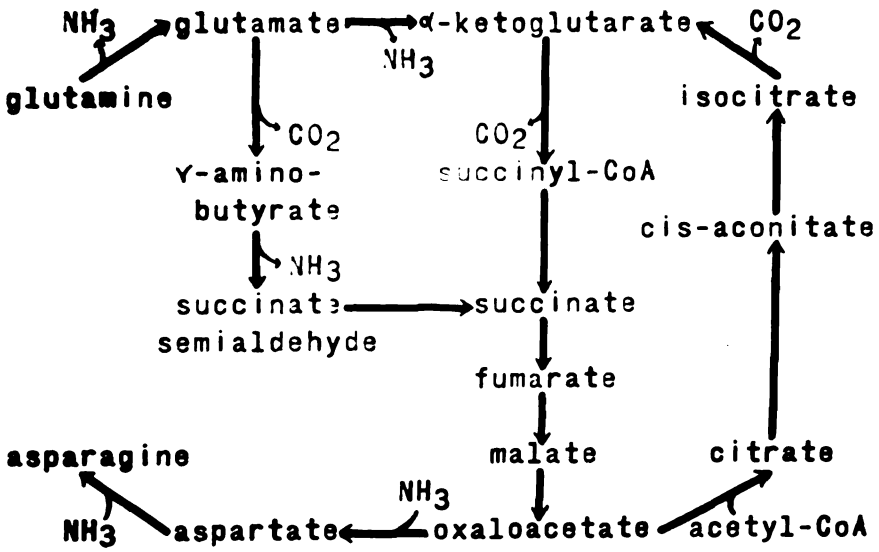


Figure 2.--The pathway of glutamine metabolism.

growth temperature and 26 C incubation temperature, there was no indication that this pathway was either stimulated or inhibited by supraoptimal temperatures (Table 10).

Since the synthesis of radioactive GAB, ASA, and ASN was relatively unaffected by changes in temperature, the percentages have been combined with CO_2 to provide a percent of total dpm isolated as products of GLA metabolism in Figure 3.

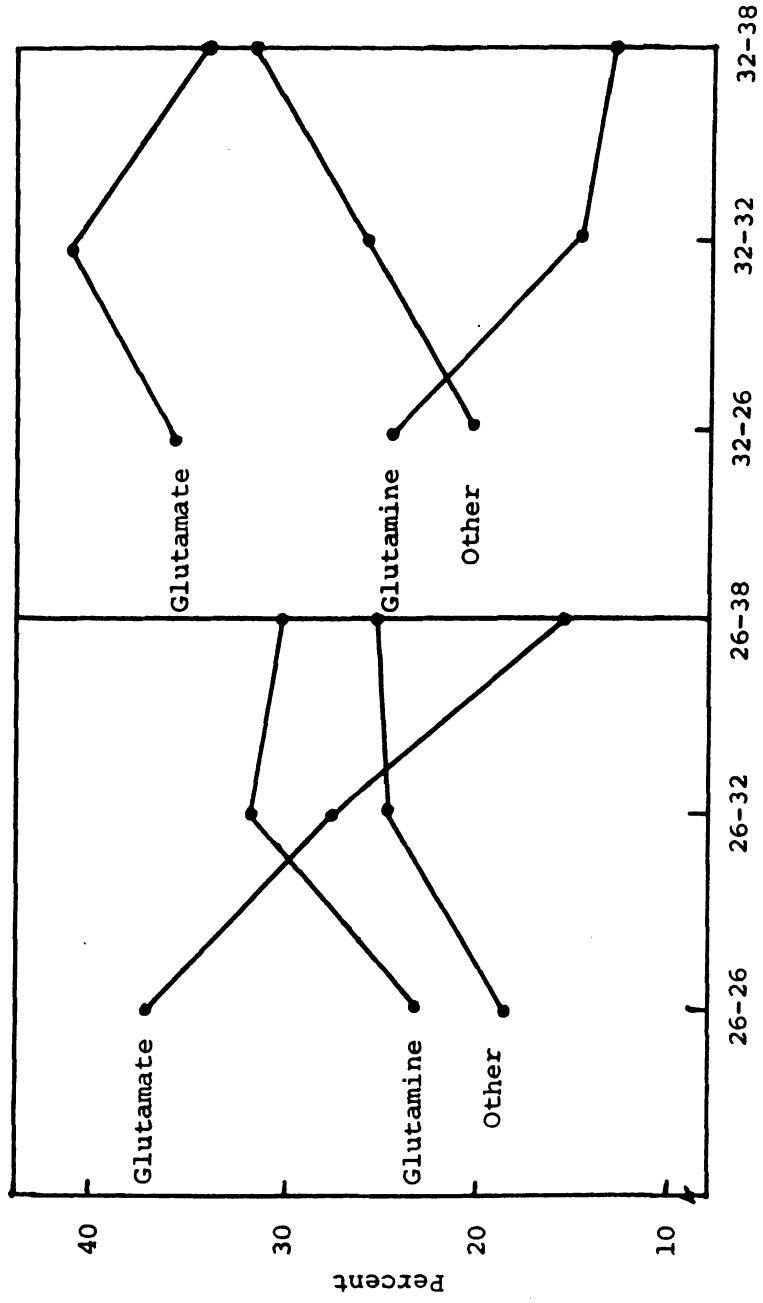


Figure 3.--The effect of temperature during growth and incubation on the percent glutamine ^{14}C. Glutamate ^{14}C and other ^{14}C-metabolites in leaf sections of Merion Kentucky bluegrass.

When Kentucky bluegrass was grown at 26 C, the increased percent GLM with increasing temperature during incubation was accompanied by a decrease in percent GLA (Figure 3). Since the radiocarbon source is GLM, there is evidence that increasing incubation temperatures block the conversion of GLM to GLA. Yet the conversion of GLA to other metabolites increased as temperatures during incubation increased. High temperatures during incubation in Table 9 resulted in the accumulation of GLM in Kentucky bluegrass leaf sections grown at 26 C. The GLA content was not affected by changes in the temperature of incubation.

When Kentucky bluegrass was grown at 32 C, GLM-^{14}C was metabolized at a higher rate during high temperature incubation periods (Figure 3). The percent GLA-^{14}C remains at a high percentage during all three temperatures of incubation, and $^{14}CO_2$ increased as temperatures during incubation were increased. In Table 9, the GLM content of the leaf tissue was reduced as temperatures during incubation were increased from 26 to 38 C, while the GLA content does not change.

When Merion Kentucky bluegrass is grown at 26 C the rate limiting reaction of glutamine metabolism appeared to be the deamination or transamination of GLM to GLA. However, when grown at 32 C the GLM pool was depleted, and $^{14}CO_2$ evolution increased sharply (Table 10).

Supraoptimal growth temperatures appear to cause the synthesis of a deaminase or transaminase isozyme which makes the carbon skeletons of GLM available for respiration.

Since depletion of the GLM pool apparently occurred through respiration, the relationship between low glutamine levels and growth stoppage at supraoptimal temperatures would most probably be in reduced synthesis of proteins and other macromolecules of which glutamine is a specific precursor.

CONCLUSIONS

Kentucky bluegrass, creeping bentgrass and bermudagrass were grown at optimal and supraoptimal temperatures. In the intermediary metabolism experiment, the effect of temperatures during growth on the uptake, respiration and incorporation into protein of four radioactive metabolites was studied. Temperature effects on growth and nitrogen content were also measured.

In the carbohydrate metabolism experiment, the effect of light duration and temperature on the carbohydrate content and glucose metabolism was investigated.

Levels of amino acids and metabolism of glutamine were measured following temperature treatments during growth and incubation of leaf sections of Merion Kentucky bluegrass in the glutamine metabolism experiment.

Based on the results, the following conclusions are drawn:

1. Increasing temperatures from 20 to 35 C decreased the dry matter production of Merion Kentucky bluegrass and Toronto creeping bentgrass and increased the dry matter production of Tifgreen bermudagrass.

2. The total nitrogen content of bluegrass and bermudagrass decreased with increasing temperatures, while no trends were evident in bentgrass.

3. Metabolic responses to high temperature stress varied with all three species.

4. Supraoptimal temperatures did not affect the quantity of glucose-^{14}C incorporated into protein. However, certain proteins necessary for growth may be limited at supraoptimal temperatures.

5. Supraoptimal growth temperatures moderate the extremes in diurnal variation in the carbohydrate content of Kentucky bluegrass and creeping bentgrass. During periods of high foliar carbohydrate levels, temperature did not affect the respiration of glucose-^{14}C. At low carbohydrate levels, supraoptimal temperatures reduced the respiration of glucose-^{14}C.

6. Glutamine was preferred over glucose as a carbon source for respiration, especially at supraoptimal temperatures for growth in Kentucky bluegrass.

7. Increasing temperatures from 26 to 38 C during incubation of Kentucky bluegrass leaf sections grown at optimum temperatures resulted in increased glutamine content and reduced glutamine metabolism. At these same temperatures of incubation, leaf sections grown at

supraoptimal temperatures resulted in depletion of the glutamine pool and increased glutamine metabolism.

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APPENDIX

APPENDIX TABLE 1.--The effect of growth temperature during a light* (5000 lux) incubation period on the ^{14}C-uptake, $^{14}CO_2$ evolved, and ^{14}C-protein synthesized from glucose-^{14}C and glutamine-^{14}C by leaf sections of Merion Kentucky bluegrass.

Source	Growth temperature (C)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	^{14}C-protein (% total)
Glucose	25	4913	4.6	1.46
	35	3344	6.6	1.76
Glutamine	25	2136	14.2	0.75
	35	2033	37.2	2.33

Values may be compared with dark incubation values for Kentucky bluegrass given for glucose and glutamine in Tables 2 and 3 respectively.

APPENDIX TABLE 2.--Total ^{14}C-uptake, $^{14}CO_2$ evolved, and protein-^{14}C synthesized from 4 radiocarbon sources by leaf sections from the youngest leaf* of Merion Kentucky bluegrass plants grown at 35 C.

Source	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	Protein-^{14}C (% total)
Glucose	769	13.6	1.66
Glutamine	987	35.3	1.19
Leucine	1280	7.3	18.73
Acetly-CoA	155	33.4	5.59

Values may be compared to those of mature leaf sections of Kentucky bluegrass grown at 35 C given in Tables 2 to 5.

APPENDIX TABLE 3.--Total ^{14}C-uptake, $^{14}CO_2$ evolved and protein-^{14}C synthesized from 4 radiocarbon sources by leaf sections of Merion Kentucky bluegrass and Toronto creeping bentgrass grown at 20 and 35 C.

Species	Source	Growth temperature (C)	Total uptake (dpm/mg dry wt/hr)	$^{14}CO_2$ (% total)	^{14}C-protein (% total)
Kentucky bluegrass	Glucose	20	3643	22.4	1.05
		30	4062	21.6	1.42
	Glutamine	20	1240	36.8	1.04
		30	2234	33.5	1.12
	Leucine	20	2327	7.1	8.72
		30	3187	4.8	9.81
	Acetyl-CoA	20	261	21.6	1.88
		30	192	17.5	2.71
Creeping bentgrass	Glucose	20	9667	26.8	1.13
		30	13616	25.1	3.26
	Glutamine	20	3795	40.8	0.78
		30	5709	52.7	1.37
	Leucine	20	4351	9.4	4.44
		30	7774	8.2	10.88
	Acetly-CoA	20	858	21.6	1.59
		30	1385	26.4	2.89