EFFECTS OF NITROGEN NUTRITION AND TEMPERATURE ON THE GROWTH, CARBOHYDRATE CONTENT, AND NITROGEN METABOLISM OF COOL - SEASON GRASSES

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ABSTRACT

EFFECTS OF NITROGEN NUTRITION AND TEMPERATURE ON THE GROWTH, CARBOHYDRATE CONTENT, AND NITROGEN METABOLISM OF COOL-SEASON GRASSES

by Harlan R. Stoin

The effects of ammonium vs. nitrate nitrogen and level of nitrogen on the growth and chemical composition of several cool-season grasses were studied at day/night temperatures of 21/16 C and 32/26 C in controlled environment chambers.

An interaction between nitrogen nutrition and temperature on the growth of the cool-season grasses occurred. In a nutrient solution experiment the growth of Italian ryegrass (Lolium multiflorum Lam.) was always reduced by the high temperature, and the reduction was greater at high levels of nitrogen and with ammonium-N. At the low temperature an increase in level of nitrate-N increased growth while an increase in level of ammonium-N decreased growth. In a soil experiment in which nitrification of added ammonium-N was not prevented, additions of either ammonium or nitrate-N caused an increase in growth of the tops of perennial ryegrass (Lolium perenne L.) and tall fescue (Festuca arundinacea Schreb.) at the low temperature but not at the high temperature. In a second soil

experiment in which nitrification of added ammonium-N was prevented, ammonium-N was superior to nitrate-N for top growth of Italian ryegrass. An increase in growth for the high level of ammonium-N but not for the high level of nitrate-N occurred at the low temperature. No response to source or level of nitrogen occurred at the high temperature.

Changes in soluble carbohydrate content did not appear to be the causal factor of growth reduction at high temperatures nor for the interactions which occurred. In no cases were the soluble carbohydrates exhausted, nor did they appear to be present in concentrations limiting to growth.

In the nutrient solution experiment protein nitrogen content appeared to be slightly increased by ammonium-N compared to nitrate-N and slightly decreased by high temperature. The soluble amino nitrogen content was generally higher with ammonium-N than nitrate-N and increased at the high temperature with ammonium-N but not with nitrate-N.

In the first soil experiment when no nitrogen was added, protein nitrogen content at the low temperature was generally lower than at the high temperature. When nitrogen was added, no differences due to temperature occurred at the first harvest. Between harvests protein nitrogen content decreased where growth continued but remained the same in the roots at the high temperature where no growth

was occurring. The soluble amino nitrogen content was generally higher with nitrogen additions and at the high temperature. The level of available nitrogen appeared to exert a greater effect than did temperature. Between harvests the soluble amino nitrogen content decreased at both temperatures. In the second soil experiment where free ammonium, nitrate, glutamine, and asparagine nitrogen were determined, these fractions all were quite low and increased with an increase in ammonium nitrogen level but not with an increase in temperature.

The effects of nitrogen nutrition and temperature on nitrogen metabolism appeared more likely to involve a blockage or slow down of protein synthesis rather than an increase in protein breakdown. An accumulation of some toxic nitrogen compound at high nitrogen levels could also be involved.

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INTRODUCTION

Temperature is a key factor in the growth of grasses whether they are grown for forage, ornamental, or recreational use. The production and vigor of the cool-season grasses are often limited by high temperatures in midsummer. Considerable information is available on the optimum temperatures for the growth of a number of cool-season grasses and their response to temperatures above the optimum. However, little is known of the actual biochemical basis for the reduction of growth of cool-season grasses at high temperatures.

Nitrogen is also an important factor in the growth of grasses. Nitrogen has a key role in plant metabolism, being a major constituent of proteins, enzymes, chlorophyll, and nucleic acids. Grasses are quite responsive to nitrogen fertilization with nitrogen fertility often becoming a limiting factor to increased grass growth. Also an interaction of temperature and nitrogen fertility on the growth response of grasses occurs. In some cases reduction in growth at high temperatures was found to be greater at high nitrogen levels than at lower levels. But again the actual biochemical basis for this interaction is unknown.

Many early workers attributed these effects to a depletion of carbohydrates. High temperatures were hypothesized to cause a reduction in carbohydrates due to a lower temperature optimum for photosynthesis than for respiration. High nitrogen levels would reduce carbohydrates by utilizing them as carbon skeletons for the formation of nitrogen-containing compounds. Although there is evidence that carbohydrates are sometimes reduced under these conditions, the evidence that carbohydrates actually become limiting to growth is inconclusive and unresolved.

Less attention has been given to the effects of environment on nitrogen metabolism. However, a number of reports have shown that various nitrogen fractions are responsive to environmental factors and thus could be involved in the biochemical reactions that bring about the reduction in grass growth at high temperatures.

The purpose of this study was to determine the effects of nitrogen source and level on the growth of cool-season grasses at the optimum temperature and at a higher temperature. Another objective was to determine what effect these variables would have on the soluble carbohydrate content and on the content of several nitrogen fractions.

REVIEW OF LITERATURE

Levitt (18) distinguishes between direct high temperature injury and indirect injury. Direct high temperature injury occurs at extremely high temperatures, occurs quickly, and usually results in death to the plant or to the affected parts of the plant. Indirect injury occurs at lower temperatures and does not cause direct kill, although the plants or plant parts may die after a prolonged period at these temperatures. The separation of these types of injury is sometimes characterized by a break in the time versus temperature curve. Levitt further divides indirect injury into metabolic high temperature injury and transpirational high temperature injury. This review will be mainly concerned with metabolic high temperature injury that occurs at temperatures above the optimum but below the point of direct high temperature kill.

The optimum temperature for growth has been defined as the highest temperature at which there is no time factor operating as distinguished from a maximum-rate temperature at which growth attains its highest rate (17). Due to environmental and genetic variables the optimum is really a range rather than a single temperature and varies for different plant parts and functions.

The optimum temperatures for growth of a number of grass species have been determined. Mitchell (19) found that the optimum temperature for daily increase in dry weight of four cool-season grasses, perennial ryegrass (Lolium perenne L.), orchardgrass (Dactylis glomerata L.), colonial bentgrass (Agrostis tenuis Sibth.), and velvetgrass (Holcus lanatus L.) was 20 C. In contrast the optimum for Dalligrass (Paspalum dilatatum Pois.), a warm-season grass, was near 30 C. Above the optimum temperatures a rapid decline in growth occurred with growth of the cool-season grasses ceasing above 35 C. The production of new tillers was also decreased above 28 C.

An optimum temperature near 20 C was reported for perennial ryegrass by Sullivan and Sprague (28) and for top growth of Kentucky bluegrass (Poa pratensis L.) by Brown (5) and Harrison (12). The optimum temperature for root and rhizome growth of Kentucky bluegrass was near 16 C.

Several workers have noted that the amount of growth reduction at temperatures above the optimum is affected by the level and form of nitrogen applied. Harrison (12) found that cutting back the leaves of non-vegetative Kentucky bluegrass plants supplied with a minus-nitrogen nutrient solution, and which had a large quantity of storage rhizomes, was less harmful during the hot summer months than was short cutting of vegetative plants which received a continuous supply of nitrogen and which had a smaller

quantity of storage rhizomes. He also found that after several defoliations, cultures at 26.4 C supplied with nitrogen produced no more top growth than minus-nitrogen cultures. In another experiment ammonium nitrogen was found to produce less growth of tops, roots, and rhizomes than did nitrate nitrogen when used in nutrient solution cultures.

Darrow (7) studied the growth of Kentucky bluegrass in sand culture with ammonium versus nitrate nitrogen, at pH's of 4.5, 5.5, and 6.5, and at soil temperatures of 15, 23, and 35 C. Growth of shoots and roots was reduced with ammonium nitrogen at all temperatures and pH's. With either nitrogen source growth was reduced at 35 C compared to the lower temperatures; however, the reduction was greater with ammonium nitrogen. The growth reductions with ammonium nitrogen were greater at low pH's.

Sprague (25) also studied the effects of ammonium versus nitrate nutrition on colonial bentgrass in sand culture. Ammonium nitrogen reduced growth of tops and roots in comparison to nitrate. He conducted no experiments in which temperatures were compared.

Pellett and Roberts (20) found that Kentucky bluegrass turf grown in solution culture at a low nitrogen level was more resistant to high temperatures than when grown at a high nitrogen level.

All of the experiments in which a nitrogen level-temperature interaction were noted were conducted with solution cultures. Comparable results from soil or field experiments have not been reported.

Many workers have attributed the reduction in growth at high temperatures to carbohydrate depletion brought about by the lower temperature optimum for photosynthesis than respiration. High nitrogen levels are also believed to reduce carbohydrates by rapidly using up the carbohydrates as carbon skeletons for nitrogen-containing compounds.

Sullivan and Sprague (28) reported the changes in carbohydrate levels in perennial ryegrass following clipping at night/day temperatures of 10/15.6, 15.6/21.1, 21.1/26.7, and 26.7/32.2 C. The sucrose content of the stubble reached a low point two weeks after clipping followed by a rise in content which was greatest at the lowest temperature treatment. The decline in sucrose in the roots lasted four weeks, followed by a rise which occurred only at the lowest temperature treatment. Reducing sugars fell more gradually after clipping and showed little response to temperature. The content of fructosan, an important reserve carbohydrate, was about 25% of the stubble dry weight at the time of clipping. Twenty-eight days after clipping, the content of fructosan had decreased to 18% at the lowest temperature treatment and 7% at the

highest temperature treatment with intermediate values for the intermediate temperatures. The content of fructosan then began increasing at the two lowest temperature treatments but continued to decrease at the two highest temperature treatments. The fructosan in the roots followed a similar pattern but at an initial level of 7%.

Differences in the dry weight production of the regrowth of tops at the different temperature treatments were noticeable two weeks after clipping. This was at a time when the content of fructosan, even at the highest temperature treatment, was still nearly 8% in the stubble and 2% in the roots. Although the decline in fructosan continued at the highest temperature treatment, complete exhaustion of carbohydrates was not observed at the termination of the experiment 40 days after clipping.

Brown (5) reported that storage of carbohydrates in Kentucky bluegrass occurred in spring and autumn when temperatures were low, but a net loss of carbohydrates occurred during late spring and summer when higher temperatures prevailed.

The application of nitrogen fertilizers has also been shown to reduce carbohydrate levels (29). However, the effect of high nitrogen levels on carbohydrates is not clearcut since high nitrogen levels have been found to increase carbohydrate levels under some conditions (4).

The evidence is not clearcut that reduced carbohydrates are the causal factor in reduced growth at high temperatures. Beinhart (3) studied the growth of white clover (Trifolium repens L.) in growth chambers and in the field. High temperatures reduced branching or production of secondary stolons, a factor closely related to continued growth and survival of white clover. During the warm months of June through September, free sugars in the stolons decreased followed by an increase in October. Branching was reduced as the decline in sugars was occurring; however, an increase in branching began again in September before the increase in sugars began. This sequence together with some unpublished growth chamber study data led the author to conclude that carbohydrate supply was not the limiting factor to summer branching of white clover.

Duff (8) found that the water soluble and alcohol soluble carbohydrates were not reduced in leaf tissue of creeping bentgrass (Agrostis palustris Huds.) grown at supraoptimal temperatures in comparison to those at the optimum and were increased at a day/night temperature of 40/30 C.

Green (10) found that carbohydrate levels were reduced at high temperatures and high nitrogen levels in four cool-season grasses, but he concluded that at no time were carbohydrate levels inadequate for growth.

Tissue culture studies have also shown that factors other than carbohydrate depletion are involved in high temperature effects. Tissue cultures of wheat (Triticum vulgare L.) and tomato (Lycopersicum esculentum L.) roots grown in media containing dextrose, a carbohydrate source, produced typical temperature versus growth curves with an optimum temperature near 30 C (34, 35).

Several workers have suggested areas other than carbohydrate depletion that may be involved in high temperature effects. Steward (27) has suggested that an important site where environmental factors may interact with metabolic processes is at the point of contact between carbohydrate and nitrogen metabolism. Specifically involved would be the keto acids, amino acids, and amides.

In a review of the biochemical aspects of temperature effects Langridge (15) listed five possible causes for high temperature effects. All ultimately involved blockage of synthesis or acceleration of breakdown of some essential metabolites. Several of the possible causes involved enzymes and/or amino acids and would thus involve protein or nitrogen metabolism.

Various nitrogen fractions have been reported to be affected by environmental factors, including temperature. Sullivan and Sprague (28), whose results with carbohydrate changes in perennial ryegrass after clipping were reported previously, also found changes in several nitrogen fractions. The 80% ethanol soluble nitrogen content in the

tops as a proportion of the total nitrogen content increased following clipping. The largest increase occurred at the highest temperature treatment. Total nitrogen in the tops declined with time after clipping but the decline was markedly less at the highest temperature treatment. Little change in total nitrogen was noted in the stubble and roots except for an increase at the highest temperature treatment. Insoluble nitrogen was also higher in both the stubble and roots at the highest temperature treatment. The authors suggested the possibility of ammonium toxicity due to a rapid digestion of proteins at high temperatures. Such a possibility had also been raised by Altergott (1), who worked at temperatures in the range of 40 to 50 C.

More recently, Petinov and Molotkovskii (21, 22) reported an accumulation of ammonia in several plant species grown at high temperatures in the range of 45-60 C. They were able to partially overcome heat injury by sprinkling plants with organic acids, which they assumed neutralized the ammonia and provided energy sources for metabolizing it. This sprinkling treatment reduced ammonia levels and increased amide levels. Respiration inhibitors were reported to decrease heat resistance. They concluded that the essence of protective reactions of plants to high temperatures is resynthesis of proteins destroyed by high temperature. The source of active metabolites and energy for resynthesis is respiration.

Beard and Daniel (2) studied the seasonal variation in the total, nonprotein, glutamine, asparagine, and total amide nitrogen fractions of Agrostis palustris Huds. leaf tissue. Temperature was found to be the major environmental factor influencing seasonal variations in these fractions. Total nitrogen content decreased at temperatures above 24 C. Glutamine showed the greatest response to temperature, dropping to very low values at soil temperatures above 24 C. Asparagine responded much less to temperature.

Steward (27) has summarized several cases where he and his co-workers noted effects of environmental factors on the level and composition of the soluble nitrogen fraction. Studies with the mint plant (Mentha piperita L.) revealed that daylight, long days, and a high K to Ca ratio in the nutrient medium tended to promote protein synthesis and glutamine accumulation in the leaves, especially at low night temperatures. In contrast, darkness, short days, a high Ca to K ratio, and high night temperatures tended to favor asparagine accumulation.

The banana (Musa species) variety Gros Michel was found to have more amide nitrogen with a higher proportion of glutamine in fruits that matured in July in Honduras than in fruits that matured in December, which had predominantly asparagine. These differences were attributed mainly to differences in night temperature.

In tulip (Tulipa gesneriana) leaves high temperatures caused an increase in serine/glycine, asparagine, glutamine, gamma-methyleneglutamic acid, and ammonia and a decrease in aspartic and glutamic acids.

In peas (Pisum sativa L.) grown under long days, the content of asparagine increased with high night temperature.

MATERIALS AND METHODS

Experiment 1. Experiment 1 was designed to determine if ammonium and nitrate nitrogen would have the same effects on growth at low and high levels in a nutrient solution and if there would be an interaction with temperature. Another objective was to determine how the soluble carbohydrate content, protein nitrogen content, and soluble amino nitrogen content would be affected by these variables.

Italian ryegrass (Lolium multiflorum Lam. var. MSU-3-LM) was grown in nutrient solution in a growth chamber at two temperatures and with two sources and two levels of nitrogen.

One-half gram of seed was planted in white quartz sand in a 32-ounce waxed cottage cheese container with drainage holes punched in the bottom. The seed was lightly covered with sand and placed in a growth chamber at a day/night temperature of 21/16 C and a light intensity of 21,500 lux. The light was provided by a combination of fluorescent and incandescent sources. The daylength was 16 hours.

The cultures were watered with tap water for 7 days at which time the young plants were about 1 cm in height.

At that time half of the cups were placed in a growth chamber at 32/26 C and the other half retained at 21/16 C. Nutrient solution treatments were initiated the same day. Two levels of nitrate nitrogen and two levels of ammonium nitrogen were compared at each temperature. The composition of the nutrient solution for each nitrogen treatment is shown in Table 1. Each treatment was duplicated. Approximately 200 ml of fresh nutrient solution was added twice daily to keep the nutrient solution composition fairly constant. Drainage from the cups indicated that this amount of nutrient solution was sufficient to saturate the sand at each watering.

TABLE 1.--The composition of the nutrient solutions used in Experiment 1.

Nutrient Solution	Millimoles/liter					
	KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	$(NH_4)_2SO_4$	$CaSO_4$	pH
Low Nitrate	2.0	1.5	2.5	--	--	7.98
High Nitrate	2.0	1.5	10.0	--	--	7.47
Low Ammonium	2.0	1.5	--	2.5	2.5	7.33
High Ammonium	2.0	1.5	--	10.0	10.0	7.80

Note: In addition to the above, each solution contained 5 mg/l of Fe as ferric citrate + 1 ml/l of a solution containing 0.6 g H_3BO_3, 0.4 g $MnCl \cdot 4H_2O$, 0.05 g $CuSO_4 \cdot 5H_2O$, and 0.02 g $H_2MoO_4 \cdot 4H_2O$ per liter.

The tops and roots were harvested 18 days after the nitrogen and temperature treatments were initiated (25 days from planting). The roots were freed of sand by thorough washing with tap water. The plant material was immediately frozen, freeze-dried, weighed, separated into tops and roots, and ground through a 20-mesh screen in a Wiley mill.

Extraction Procedure. The ground, dried plant material was extracted with 70% ethanol as follows: Twenty ml of 70% ethanol was added to a 0.5 g sample of ground tissue in a 50 ml centrifuge tube. The tube was stoppered and shaken 15-18 hours, centrifuged at 35000 g for 10 minutes, and the extract decanted through a funnel containing Whatman #1 filter paper into a 100 ml volumetric flask. An additional 20 ml of 70% ethanol was added to the residue in the centrifuge tube and then shaken an additional 3-4 hours. Next the uncentrifuged mixture was poured with washing into the same filter paper and flask as used before. The residue in the filter paper was washed with 4 additional 10 ml portions of 70% ethanol. The extract in the volumetric flask was now made up to volume with 70% ethanol. The soluble amino nitrogen and soluble carbohydrate determinations were made on appropriate dilutions of this extract. The residue in the filter paper was saved for the protein nitrogen analysis.

Soluble Carbohydrate Determination. Alcohol soluble carbohydrates were determined by the phenol-sulfuric acid colorimetric method (13) on a 1/10 dilution with 70% ethanol of the above extract. One ml of the diluted extract was placed in a matched test tube and 1 ml of 5% phenol added. Then 5 ml of concentrated sulfuric acid was added rapidly to ensure mixing of air into the solution. The tube was allowed to stand 15 minutes, shaken, and then allowed to cool to room temperature. The color density in the tube was read at a wavelength of 490 mu with a Bausch and Lomb Spectronic 20 spectrophotometer and compared with a sucrose standard curve.

Soluble Amino Nitrogen Determination. The soluble amino nitrogen content was determined on a 1/10 dilution with 70% ethanol of extract by the ninhydrin colorimetric method of Rosen (23). The following reagents were used:

1. Stock NaCN: 0.01 M (490 mg/l).
2. Acetate buffer: 3600 g NaOAc · $3H_2O$ + 5 l H_2O + 675 ml glacial acetic acid. Make up to 10 l with H_2O. Adjust pH to 5.3 to 5.4 with sodium hydroxide or acetic acid.
3. Acetate-cyanide: 0.0002 M NaCN in acetate buffer; 2 ml of Reagent 1 made up to 100 ml with Reagent 2. This reagent is unstable and must be used within 4 hours of mixing (16).
4. Ninhydrin: 3% in Methyl Cellosolve (peroxide free).
5. Diluent: isopropyl alcohol:water (1:1).

One ml of the diluted extract was placed in a matched test tube and 1/2 ml of Reagent 3 and 1/2 ml of Reagent 4 were added. The mixture was then heated for 15 minutes in a boiling water bath. Immediately after removal from the water bath, 5 ml of diluent (Reagent 5) was rapidly added and the tube vigorously shaken. The tube was allowed to cool to room temperature and the color density read at a wavelength of 570 mu with a Bausch and Lomb Spectronic 20 spectrophotometer. The color density was compared with a glutamic acid standard curve. If the optical density of a tube was too high to give a good reading, further 5 ml portions of isopropyl-water diluent were added until the density was below 0.75.

Protein Nitrogen Determination. Protein nitrogen was determined on the residue from the alcohol extraction. The residue and the filter paper containing it were dried and placed in a 125 ml Erlenmeyer flask. Fifty ml of 3 N HCl was added and the mixture autoclaved for 6 hours at 120 C and 15 lbs pressure to hydrolyze the protein to amino acids (16). The mixture was then cooled, made up to 200 ml in a volumetric flask, the solids allowed to settle out overnight, and 1 ml of the solution diluted to 10 ml with water. The amino nitrogen content of 1 ml of the diluted solution was determined by Rosen's method. The amino nitrogen content was equated with protein nitrogen content.

Experiment 2. Experiment 2 was designed to determine whether there would be an interaction of nitrogen nutrition and temperature on the growth and chemical composition of grasses grown in soil.

Two cool-season grass species, perennial ryegrass and tall fescue (Festuca arundinacea Schreb. var. Kentucky 31) were grown in soil in a growth chamber. The grasses were grown at two temperatures and with three nitrogen fertility treatments.

One-half gram of seed was planted in Conover sandy loam soil in a 32-ounce waxed cottage cheese container with drainage holes punched in the bottom. The seed was lightly covered with soil and watered. The cups were placed in a growth chamber at 21/16 C day/night temperature and 21,5000 lux light intensity. The light was provided by a combination of fluorescent and incandescent sources. The daylength was 16 hours. After 10 days, when the young plants were 1-2 cm tall, half of the cups were placed in a growth chamber at 32/26 C and half retained at 21/16 C. Four days later three fertility treatments were applied. The first treatment consisted of no additional nitrogen, the second treatment was the addition of 70 mg of nitrogen per culture as $Ca(NO_3)_2$, and the third treatment was the addition of 70 mg of nitrogen as $(NH_4)_2SO_4$. Nothing was added to prevent nitrification of the added ammonium nitrogen. Each treatment

was replicated 3 times. The cultures were kept well supplied with water.

Eighteen days after the temperature treatments were initiated (28 days after planting), the top growth above 4 cm was harvested from 2 replications of each treatment. The tops and roots were harvested from the third replication. The soil was washed from the roots with tap water. The plant samples were immediately frozen, freeze-dried, and ground through a 20-mesh screen with a Wiley mill.

The 2 replications from which only the tops were harvested were allowed to regrow for 11 days, and then both the tops and roots from these plants were harvested and treated as above.

The extraction procedure and chemical determinations for this experiment were the same as outlined for Experiment 1 except that for the soluble amino nitrogen determination 1/2 ml of the undiluted extract to which 1/2 ml of H_2O was added was used instead of 1 ml of the 1/10 diluted extract. This was necessary due to the lower content of soluble amino nitrogen in Experiment 2 compared to Experiment 1.

Experiment 3. Experiment 3 was designed to determine if the effects of ammonium and nitrate nitrogen on growth and chemical composition of grasses and their interaction with temperature would be the same with grasses grown in soil

in which the nitrification of ammonium nitrogen was inhibited.

Italian ryegrass was grown in a soil-sand mixture in a growth chamber at two temperatures and with two sources and two levels of nitrogen.

One-half gram of seed was planted as in Experiment 2. The cultures were placed in the greenhouse for 6 days and then moved into growth chambers at 21/16 C day/night temperatures and 21,500 lux light intensity. The light was provided by a combination of fluorescent and incandescent sources. The daylength was 16 hours. Three days later, when the young plants were 2 to 3 cm in height, the temperature in one chamber was raised to 32/26 C and the other retained at 21/16 C. Nutrient treatments consisting of ammonium nitrogen versus nitrate nitrogen and at two levels of each source were applied the same day. The treatments are shown in Table 2. The N-Serve (2-chloro-6-(trichloromethyl pyridine)) (Dow Chemical Company) added to the ammonium treatments served to prevent nitrification of the added ammonium sulfate by selectively inhibiting nitrifying bacteria (9). Two weeks later the nitrogen treatments were repeated but with no addition of the other nutrients or N-Serve. Each treatment was replicated 3 times. The cultures were kept well supplied with water.

Twenty-seven days after the temperature treatments were initiated (36 days from planting), the top growth was harvested at ground level and frozen immediately. Extraction of the samples was done on undried samples.

Extraction procedure. A small portion of the frozen samples was weighed and dried overnight at 90 C to determine moisture content and dry weight. The dried sample was cut into small pieces and used for the total amino nitrogen determination.

The remainder of the frozen sample was allowed to thaw and then placed in 2 one-quart polyethylene freezer bags. The sample in the bags was then pressed between two blocks of wood in a vise. The plant juice ran out the opening of the bags into a centrifuge tube. The juice so collected was centrifuged at 35000 g for 10 minutes.

One ml of the cleared juice was pipetted into a weighing dish, weighed, and dried at 90 C to determine soluble solids content and to provide a value for adjustment of chemical composition figures for the juice to a dry weight basis.

This method of pressing the juice out of a previously frozen plant sample has been shown to give a sample of uniform composition comparable to extracts from dried material (6, 24).

The cleared juice sample was heated rapidly to 75 C on a steam bath and maintained between 75-80 C for 10

TABLE 2.--Nutrients added for the various treatments of Experiment 3.

Fertility Treatment	Millimoles/Culture				
	$CaHPO_4$	KCl	$MgSO_4$	$Ca(NO_3)_2$	$(NH_4)_2SO_4$
+Low Nitrate	3.33	3.33	1.67	2.5 (72 mg N)	--
+High Nitrate	3.33	3.33	1.67	5.0 (140 mg N)	--
+Low Ammonium	3.33	3.33	1.67	--	2.5 (70 mg N)
+High Ammonium	3.33	3.33	1.67	--	5.0 (140 mg N)

Note: The first three salts were applied only once. The nitrogen treatments were applied twice. One hundred ml of a 20 ppm solution of N-Serve was added per pot to the ammonium treatments to prevent nitrification.

minutes to precipitate soluble proteins (26). Appropriate dilutions of this deproteinated extract were used for determinations of the soluble constituents.

The water soluble carbohydrates and soluble amino nitrogen were determined by the method outlined in Experiment 1.

Free Ammonium Nitrogen Determination. Free ammonium nitrogen was determined colorimetrically with Nessler reagent (Sargent Chemical Co.) after the free ammonium ions were separated from the extract on a 5 X 0.9 cm column of AG 50-X-4 cation exchange resin, 200-400 mesh in the sodium form (Calbiochem). Separation of the ammonium ions from the extract was necessary because of substances in the pure extract which interfered with direct Nesslerization.

The exchange column was poured with AG 50-X-4 in the H^+ form and then converted to the Na^+ form by passing 10 to 15 ml of 2 N NaOH through the column with 4 to 6 lbs. of pressure, followed by washing with distilled water. A 1 ml sample of the undiluted, deproteinated extract was passed into the column and washed with several portions of distilled water. The ammonium ions were retained on the column. Elution of the ammonium ions was performed by passing 10 ml of 1.5 N NaOAC through the column, also with 4 to 6 lbs. of pressure. The eluate was collected in a 50 ml volumetric flask.

Following elution, the solution in the 50 ml volumetric flask, which contained the ammonium ions, was made almost to volume with distilled water. Then 1 ml of Nessler reagent was added and the mixture was shaken and allowed to stand 15 minutes. After 15 minutes the solution was made up to volume and a portion poured into a matched test tube. The color density was read at a wavelength of 410 mu with a Bausch and Lomb Spectronic 20 spectrophotometer. The optical density was compared with an NH_4Cl standard curve. In order to prepare a standard comparable to the eluate solutions, it was necessary to add 10 ml of 1.5 N NaOAC to the flasks in which the NH_4Cl standards were run since the NaOAC in the eluate solutions produced a slight cloudiness and thus increased the optical density in comparison to ammonium ions in water.

Glutamine, Asparagine, and Nitrate Nitrogen Determinations. Glutamine, asparagine, and nitrate nitrogen were determined by a combination and modification of the methods of Vickery et al. (33) and Varner et al. (32).

In order to hydrolyze the amide group of glutamine to ammonia without hydrolyzing the asparagine, 1 ml of deproteinated extract was placed in a 50 ml Erlenmeyer flask and 3 ml of a phosphate-borate buffer at pH 6.5 (10.2 g KH_2PO_4 and 4.8 g $Na_2B_4O_7$ per 1.) was added and the mixture heated for two hours on a steam bath (33).

To prevent evaporation and loss of ammonia, the flask was stoppered with a one-hole rubber stopper into which a 1 ml transfer pipet was fitted.

Following heating, a few drops of water were washed down the pipet to return any volatilized ammonia to the flask. The contents of the flask were cooled and then transferred with washing to the distillation flask of a micro-Kjeldahl distillation unit. Ten ml of a saturated $Na_2B_4O_7$ solution adjusted to a pH of 10 with 40% NaOH was added to make the contents slightly alkaline. The ammonia was then steam distilled into a receiving flask containing 10 ml of 1% boric acid plus a bromcresol green and methyl red indicator (20 g boric acid + 10 ml of a 0.033% bromcresol green and 0.066% methyl red in methanol solution made up to 2 l with water). Distillation was continued 5 minutes. The ammonia thus collected consisted of that from free ammonium and glutamine nitrogen.

Next, 10 ml of 40% NaOH was added to the distillation flask, a new receiving flask containing 10 ml of boric acid and indicator was put in place, and steam distillation continued for 3 minutes. The ammonia now collected was from the hydrolysis of the amide group of asparagine under these strongly alkaline conditions (32).

Nitrate nitrogen was also determined on the same sample after the nitrate was reduced to ammonia (32). First glucose interference was removed by adding 2 ml of

15% tartaric acid and 1/2 ml of 25% cupric sulfate to the distillation flask. Then the mixture was steam heated for 3 minutes. Next, a new receiving flask containing 1% boric acid and indicator was put in place and 5 ml of 20% ferrous sulfate was added to the distillation flask. The ferrous sulfate reduced the nitrate to ammonia, which was steam distilled into the receiving flask for 3 minutes. This procedure also reduces nitrite to ammonia, but nitrite is usually present only in very small quantities in plant tissue.

The boric acid in the receiving flasks was titrated with 0.0076 N HCl to a pink end point. A blank was run through all three distillations and the value of the blank subtracted from the sample values.

Total Amino Nitrogen Determination. Total amino nitrogen was determined on an over-dried sample which had been cut into fine pieces. About 100 mg of dried sample was placed in a 100 ml volumetric flask and 10 ml of 4 N HCl added. The flask was then autoclaved at 120 C and 15 lbs. pressure to hydrolyze the protein to amino acids. After 1 hour the flask was removed from the autoclave, the contents were swirled around to wash down the sides, and then autoclaved for three more hours. Next, 10 ml of additional 4 N HCl was added to wash the sides down, and autoclaving was continued for two additional hours. Following autoclaving, the flask was filled to volume with water and the solids

allowed to settle out overnight. The amino nitrogen content of a one ml sample of a 1/10 dilution with water of this solution was then determined by Rosen's method as outlined for Experiment 1. Amide nitrogen, which is hydrolyzed to ammonium nitrogen by this process, is also included in the amino nitrogen determination.

RESULTS

The results of the experiments were analyzed statistically as randomized block experiments. The treatment means were separated by using Duncan's multiple range test. Means in the tables followed by an identical letter do not differ at the 5% level of significance. The tops and roots were analyzed separately.

Experiment 1. The growth of Italian ryegrass in nutrient solution was affected by nitrogen source, nitrogen level, and temperature (Table 3). Significant interactions among the three variables also occurred.

TABLE 3. The effects of nitrogen nutrition and temperature on the growth of Italian ryegrass grown in sand culture (means of 2 replications).

Nutrient Solution Variable	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	g/pot		g/pot	
Low Nitrate	2.5de	1.5d	1.1c	1.0c
High Nitrate	3.2f	2.6e	0.7b	0.6b
Low Ammonium	2.6e	1.5d	0.7b	0.5b
High Ammonium	2.4d	1.0c	0.1a	0.1a

At the low temperature and low nitrogen level nitrate-N was equal to ammonium-N as a nitrogen source. However, nitrate-N increased growth whereas ammonium-N decreased growth at the high level of nitrogen. Tops and roots reacted similarly.

Growth of tops and roots was reduced for all nitrogen treatments at the high temperature in comparison to the same treatment at the low temperature. This reduction was greater at high nitrogen levels and was greater with ammonium-N than with nitrate-N.

Soluble Carbohydrate Content. The effects of nitrogen treatment and temperature on the 70% ethanol soluble carbohydrate content are shown in Table 4.

TABLE 4. The effects of nitrogen nutrition and temperature on the 70% ethanol soluble carbohydrate content of Italian ryegrass grown in sand culture (means of 2 replications).

Nutrient Solution Variable	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	mg/g DW		mg/g DW	
Low Nitrate	89bc	46cd	92cd	34ab
High Nitrate	100d	46cd	76a	40bcd
Low Ammonium	90bc	48d	87bc	37bc
High Ammonium	81ab	43bcd	94cd	26a

At the low temperature the only nutritional response found was a small increase in carbohydrate in the tops for the high nitrate-N treatment. High temperature caused no change in the carbohydrate content in the tops for the low level of either nitrate-N or ammonium-N. However, the content for the high nitrate-N treatment was lower at the high temperature than at the low temperature and lower than for the other nitrogen treatments at the high temperature. The content for the high ammonium-N treatment was higher at the high temperature than at the low temperature.

Protein Nitrogen Content. The effects of nitrogen treatment and temperature on protein content are shown in Table 5.

TABLE 5. The effects of nitrogen nutrition and temperature on the protein nitrogen content of Italian ryegrass grown in sand culture (means of 2 replications).

Nutrient Solution Variable	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	mg N/g DW		mg N/g DW	
Low Nitrate	23.30abc	12.72cd	20.16ab	8.24ab
High Nitrate	25.54abc	10.02abc	19.26a	10.56bc
Low Ammonium	27.33bc	14.14d	23.30abc	12.72cd
High Ammonium	30.02c	17.25e	19.75a	7.47a

No differences in the tops due to nitrogen treatment were found at either temperature. High temperature did cause a reduction in protein nitrogen content with the high ammonium-N treatment, but no reduction occurred for the other treatments, although the content for the low and high nitrate-N treatments was lower than for the high ammonium-N treatment, and the high nitrate-N treatment was also lower than for the low ammonium-N treatment.

In the roots the protein nitrogen content was always lower than in the tops. It was higher for the high ammonium-N treatment at the low temperature. The protein nitrogen content for the low ammonium-N treatment was significantly higher than for the high nitrate-N treatment but not significantly higher than for the low nitrate-N treatment. At the high temperature the protein nitrogen content of the roots was lower compared to the content at the low temperature for the low nitrate-N and high ammonium-N treatments but not for the high nitrate-N and low ammonium-N treatments. At the high temperature the protein nitrogen content was also higher for the low ammonium-N treatment than for the low nitrate-N treatment but was lower for the high ammonium-N treatment than for the high nitrate-N treatment.

Soluble Amino Nitrogen Content. The effects of nitrogen treatment and temperature on the soluble amino nitrogen content are shown in Table 6.

TABLE 6.--The effects of nitrogen nutrition and temperature on the soluble amino nitrogen content of Italian ryegrass grown in sand culture (means of 2 replications).

Nutrient Solution Variable	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	mg N/g DW		mg N/g DW	
Low Nitrate	2.60a	1.24a	2.06a	1.12a
High Nitrate	2.59a	0.94a	2.77a	1.48a
Low Ammonium	4.65b	5.36bc	6.28c	5.90c
High Ammonium	7.73d	5.18b	20.16e	9.78d

No differences due to nitrogen level or temperature occurred for the nitrate-N treatments. The content of soluble amino nitrogen in both the tops and roots was much higher with ammonium-N than with nitrate-N. Also the high level of ammonium-N and the high temperature increased the soluble amino nitrogen content of the tops. The only increase in the roots due to ammonium level and temperature occurred for the high ammonium-N treatment at the high temperature. The increases with ammonium-N compared to nitrate-N were greater in the roots than in the tops except for the ammonium-N treatment at the high temperature.

Experiment 2. The growth of perennial ryegrass and tall fescue in soil was also affected by additions of nitrogen and by temperature. A significant interaction between nitrogen fertility and temperature also occurred. The dry weight production above 4 cm 28 days after planting is shown in Table 7. The regrowth above 4 cm after 10 days is shown in Table 8. The dry weights of the roots for the one replication harvested at the first date are shown in Table 7 and for the two replications harvested at the second date in Table 8.

At the low temperature there was an increase in growth of the tops with applied nitrogen. Either source of nitrogen gave the same response. The same was true for the regrowth at low temperatures. There were no differences between species.

At the high temperature there was no response to added nitrogen of either source at the first harvest nor for regrowth. With no added nitrogen, the growth of tops at the high temperature in comparison to the low temperature was the same for the tall fescue and only slightly reduced for the perennial ryegrass. However, growth of tops was always significantly reduced by the high temperature for treatments which received added nitrogen. There were no differences due to species or nitrogen source.

The roots showed no response to added nitrogen at either temperature but were always greatly reduced by the

TABLE 7.--The effects of added nitrogen and temperature on the growth of perennial ryegrass and tall fescue grown in soil in a growth chamber. First harvest.

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops[1]	Roots[2]	Tops[1]	Roots[2]
	g/pot		g/pot	
Lolium perenne				
No added N	1.0c	1.5	0.8ab	0.5
+Ammonium N	1.3d	1.3	0.8abc	0.7
+Nitrate N	1.3d	1.2	0.9bc	0.8
Festuca arundinacea				
No added N	0.8abc	1.8	0.7a	0.8
+Ammonium N	1.4d	1.3	0.8abc	0.7
+Nitrate N	1.5d	1.5	0.8abc	0.6

[1]Topgrowth above 4 cm. Means of 3 replications.

[2]Values of one replication.

TABLE 8.--The effects of added nitrogen and temperature on the regrowth and root production of perennial ryegrass and tall fescue grown in soil in a growth chamber. Second harvest (means of 2 replications).

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Regrowth above 4cm	Roots	Regrowth above 4cm	Roots
	g/pot		g/pot	
Lolium perenne				
No added N	0.4bc	2.0b	0.2a	0.8a
+Ammonium N	0.5cd	2.3bc	0.2a	0.8a
+Nitrate N	0.6d	2.4c	0.2a	0.7a
Festuca arundinacea				
No added N	0.3ab	2.2bc	0.2a	0.8a
+Ammonium N	0.5cd	2.0b	0.2a	0.8a
+Nitrate N	0.6d	2.0b	0.3ab	0.8a

high temperature. There were no species differences. Although only one sample of roots was available at the first harvest, the roots appeared to continue growing between harvests at the low temperature but grew very little, if at all, between harvests at the high temperature.

Alcohol Soluble Carbohydrate Content. The effects of added nitrogen and temperature on the 70% ethanol soluble carbohydrate content are shown for the first harvest in Table 9 and for the second harvest in Table 10.

The 70% ethanol soluble carbohydrate content did not vary much between treatments or harvest dates. It was always much lower in the roots than in the tops. A few differences occurred but did not seem related to any particular temperature or fertility treatment except that the carbohydrate content may have been slightly lower at the high temperature with added nitrogen for the first harvest of tall fescue. This difference did not occur at the second harvest.

Protein Nitrogen Content. The effects of added nitrogen and temperature on the protein nitrogen content are shown for the first harvest in Table 11 and the second harvest in Table 12.

At the first harvest and the low temperature the protein nitrogen content of the tops and roots was higher with added nitrogen. At the high temperature there may

TABLE 9.--The effects of added nitrogen and temperature on the 70% ethanol soluble carbohydrate content of perennial ryegrass and tall fescue grown in soil in a growth chamber. First harvest.

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops[1]	Roots[2]	Tops[1]	Roots[2]
	mg/g DW		mg/g DW	
Lolium perenne				
No added N	101ab	42	106bc	55
+Ammonium N	86ab	44	104bc	44
+Nitrate N	102bc	51	81a	51
Festuca arundinacea				
No added N	111c	32	103bc	76
+Ammonium N	108c	44	77a	43
+Nitrate N	110c	36	79a	57

[1]Means of 3 replications.

[2]Values of one replication.

TABLE 10.--The effects of added nitrogen and temperature on the 70% ethanol soluble carbohydrate content of perennial ryegrass and tall fescue grown in soil in a growth chamber. Second harvest (means of 2 replications).

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	mg/g DW		mg/g DW	
Lolium perenne				
No added N	83a	34a	97abc	46bcde
+Ammonium N	95abc	39abc	94abc	39abc
+Nitrate N	103abc	40abcd	97abc	43abcde
Festuca arundinacea				
No added N	99abc	48cde	98abc	50e
+Ammonium N	108bc	50e	104abc	50e
+Nitrate N	115c	36ab	91ab	42abcde

TABLE 11.--The effects of added nitrogen and temperature on the protein nitrogen content of perennial ryegrass and tall fescue grown in soil in a growth chamber. First harvest.

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops[1]	Roots[2]	Tops[1]	Roots[2]
	mg N/g DW		mg N/g DW	
Lolium perenne				
No added N	12.64a	7.54	14.76ab	11.19
+Ammonium N	18.07bc	10.60	18.33bc	11.54
+Nitrate N	20.16c	11.31	19.26c	12.25
Festuca arundinacea				
No added N	13.49a	6.60	18.88bc	11.31
+Ammonium N	17.97bc	10.13	18.29bc	11.31
+Nitrate N	18.00bc	8.95	20.31c	10.84

[1]Means of 3 replications.

[2]Values of one replication.

TABLE 12.--The effects of added nitrogen and temperature on the protein nitrogen content of perennial ryegrass and tall fescue grown in soil in a growth chamber. Second harvest (means of 2 replications).

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	mg N/g DW		mg N/g DW	
Lolium perenne				
No added N	9.54ab	6.72ab	11.42abc	9.30cd
+Ammonium N	11.78abc	6.83ab	13.31ab	10.60d
+Nitrate N	11.78abc	7.66bc	12.02abc	10.13d
Festuca arundinacea				
No added N	8.72a	5.18a	12.48bc	10.02d
+Ammonium N	11.78abc	6.95ab	12.72bc	11.19d
+Nitrate N	11.19abc	7.07b	12.48bc	10.48d

have been a small increase in the tops with added nitrogen for perennial ryegrass but not for tall fescue. No change in protein nitrogen content occurred in the roots due to added nitrogen at the high temperature. However, the protein nitrogen content of the roots was higher at the high temperature than at the low temperature when no nitrogen was added but was not different for treatments receiving added nitrogen. The protein nitrogen content between species did not differ except for the one case noted above.

By the time of the second harvest, the protein nitrogen levels had decreased from the first harvest for all treatments except for the roots at the high temperature. The differences due to added nitrogen at the low temperature were now no longer statistically significant. The protein nitrogen contents of the tops decreased to the same levels at both temperatures, but in the roots the protein nitrogen levels decreased to such an extent at the low temperature than they were now significantly lower than at the high temperature. There were no differences between species at the second harvest.

Soluble Amino Nitrogen Content. The effects of added nitrogen and temperature on the soluble amino nitrogen content are shown for the first harvest in Table 13 and for the second harvest in Table 14.

TABLE 13.--The effects of added nitrogen and temperature on the soluble amino nitrogen content of perennial ryegrass and tall fescue grown in soil in a growth chamber. First harvest.

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops[1]	Roots[2]	Tops[1]	Roots[2]
	mg N/g DW		mg N/g DW	
Lolium perenne				
No added N	1.01ab	0.94	1.43cd	1.20
+Ammonium N	1.29bc	1.18	1.78def	1.34
+Nitrate N	1.83ef	1.18	1.82ef	1.32
Festuca arundinacea				
No added N	0.90a	0.90	1.47cde	1.41
+Ammonium N	1.38bc	0.90	1.82ef	1.77
+Nitrate N	1.37bc	0.78	1.99f	1.88

[1]Means of 3 replications.

[2]Values of one replication.

TABLE 14.--The effects of added nitrogen and temperature on the soluble amino nitrogen content of perennial ryegrass and tall fescue grown in soil in a growth chamber. Second harvest (means of 2 replications).

Fertility Treatment	Day/Night Temperature			
	21/16 C		32/26 C	
	Tops	Roots	Tops	Roots
	mg N/g DW		mg N/g DW	
Lolium perenne				
No added N	0.56ab	0.28b	0.78cd	0.67c
+Ammonium N	0.67bc	0.33b	1.12e	0.88d
+Nitrate N	0.65bc	0.27b	1.26f	0.88d
Festuca arundinacea				
No added N	0.46a	0.15a	0.90d	0.78d
+Ammonium N	0.60ab	0.26b	1.26f	1.06e
+Nitrate N	0.62b	0.22ab	1.44g	0.86d

For the first harvest, added nitrogen caused an increase in soluble amino nitrogen content of the tops and roots at both temperatures except for the added ammonium-N treatment of perennial ryegrass at the low temperature and the roots of tall fescue at the low temperature. The content was always higher at the high temperature in comparison to the same treatment at the low temperature except for the added nitrate-N of perennial ryegrass. The soluble amino nitrogen content of the roots was generally the same or less than that of the tops.

At the second harvest added nitrogen caused an increase in soluble amino nitrogen content of the tops and roots except for the roots of perennial ryegrass at the low temperature and the roots of tall fescue for the added nitrate-N treatment at the high temperature. The soluble amino nitrogen content had considerably decreased between harvests for all treatments, more at the low temperature than at the high temperature. It was always lower in the roots than the tops.

Experiment 3. The growth of annual reygrass in a soil-sand mixture was also affected by nitrogen source and level and by temperature (Table 15). Significant interactions among the variables also occurred.

The top growth with ammonium-N was superior to that with nitrate-N at the low temperature. A response

to the high level of nitrogen occurred with the addition of ammonium-N but not with nitrate-N. At the high temperature no differences due to added nitrogen occurred.

TABLE 15.--The effects of nitrogen nutrition and temperature on the top growth and water soluble carbohydrate content of the tops of Italian ryegrass grown in a soil-sand mixture in a growth chamber (means of 3 replications).

Fertility Treatment	Dry Weight		Soluble Carbohydrate	
	21/16 C	32/26 C	21/16 C	32/26 C
	g/pot		g/pot	
+Low Nitrate N	2.9a	3.5abc	194c	176bc
+High Nitrate N	3.9abc	3.3ab	220c	140ab
+Low Ammonium N	4.7c	3.6abc	213c	142ab
+High Ammonium N	6.7d	4.4bc	178bc	121a

There was little difference between temperature treatments with added nitrate-N, but growth was significantly reduced by the high temperature with added ammonium-N. Even though no differences in dry weight production due to temperature occurred for some treatments, the grass was always a darker, bluish-green color with more dead leaves and leaf tips evident at the high temperature.

Water Soluble Carbohydrate Content. The effects of the nitrogen treatments and temperature on the water soluble carbohydrate content are shown in Table 15.

No differences in soluble carbohydrate content due to nitrogen treatments occurred at either temperature. However, the soluble carbohydrate content was lower at the high temperature for all nitrogen treatments except the low nitrate-N treatment.

The total soluble solids (Table 16) also showed a decrease at the high temperature. A comparison of the total soluble solids with the soluble solids minus water soluble carbohydrates indicated that the temperature responses of the total soluble solids were due to the water soluble carbohydrate fraction.

Total Amino Nitrogen and Protein Nitrogen Content. The total amino nitrogen and protein nitrogen contents were highly variable and no definite trend due to treatments was found (Table 17).

Soluble Nitrogen Fractions. Several of the soluble nitrogen fractions were responsive to nitrogen treatments and/or temperature.

The soluble amino nitrogen was quite variable in this experiment (Table 18). At both temperatures the high level of ammonium-N or nitrate-N increased the soluble amino nitrogen content but the content did not differ at

TABLE 16.--The effects of nitrogen nutrition and temperature on the soluble solids in the tops of Italian ryegrass grown in a soil-sand mixture in a growth chamber (means of 3 replications).

Fertility Treatment	Total Soluble Solids		Soluble Solids Minus Water Soluble Carbohydrates	
	Day/Night Temperature			
	21/16 C	32/26 C	21/16 C	32/26 C
	mg/g DW		mg/g DW	
+Low Nitrate N	398b	331a	204a	155a
+High Nitrate N	398b	327a	177a	187a
+Low Ammonium N	387b	323a	173a	182a
+High Ammonium N	391b	295a	213a	174a

TABLE 17.--The effects of nitrogen nutrition and temperature on the total amino nitrogen and protein nitrogen content of the tops of Italian ryegrass grown in a soil-sand mixture in a growth chamber (means of 3 replications).

Fertility Treatment	Total Amino N		Protein N[1]	
	Day/Night Temperature			
	21/16 C	32/26 C	21/16 C	32/26 C
	mg N/g DW		mg N/g DW	
+Low Nitrate N	7.83a	8.76abc	7.30a	8.10ab
+High Nitrate N	8.63ab	12.01abc	7.93ab	10.97ab
+Low Ammonium N	9.92abc	13.42c	9.38ab	12.57b
+High Ammonium N	12.62bc	11.53abc	11.01ab	9.73ab

[1]Protein nitrogen was calculated as the total amino nitrogen minus the various soluble nitrogen fractions except nitrate nitrogen.

TABLE 18.--The effects of nitrogen nutrition and temperature on the soluble amino nitrogen fraction in the tops of Italian ryegrass grown in a soil-sand mixture in a growth chamber (means of 3 replications.)

Fertility Treatment	Soluble Amino N		Soluble Amino N Minus Glutamine and Asparagine N	
	Day/Night Temperature			
	21/16 C	32/26 C	21/16 C	32/26 C
	mg N/g DW		mg N/g DW	
+Low Nitrate N	0.41a	0.55ab	0.30a	0.47bc
+High Nitrate N	0.60abc	0.82c	0.53cd	0.68d
+Low Ammonium N	0.43ab	0.69bc	0.35ab	0.56cd
+High Ammonium N	1.21d	1.44d	0.84e	1.11f

the high temperature in comparison to the same nitrogen treatment at the low temperature.

If the amino nitrogen fraction contributed by the amides glutamine and asparagine is subtracted from the total soluble amino nitrogen, an increase at the high temperature is evident with the exception of the high nitrate-N treatment (Table 18).

The levels of free ammonium and nitrate nitrogen in the plant tissue were quite low and quite variable for all treatments (Table 19). Both of these fractions generally increased for the high level of nitrogen at both temperatures although there were a few exceptions. Temperature had no significant effect on these fractions except for a slight increase in free ammonium nitrogen at the high temperature for the low ammonium-N treatment.

Glutamine and asparagine nitrogen levels were also quite low and quite variable (Table 20). A large increase in both of these amides did occur for the high ammonium treatment at both temperatures. No significant differences due to temperature treatment occurred.

TABLE 19.--The effects of nitrogen nutrition and temperature on the free ammonium nitrogen and nitrate nitrogen fractions in the tops of Italian ryegrass grown in a soil-sand mixture in a growth chamber (means of 3 replications).

Fertility Treatment	Free Ammonium N		Nitrate N	
	Day/Night Temperature			
	21/16 C	32/26 C	21/16 C	32/26 C
	ug N/g DW		ug N/g DW	
+Low Nitrate N	26a	25a	11a	12a
+High Nitrate N	26a	37b	9a	27a
+Low Ammonium N	30ab	34ab	11a	25a
+High Ammonium N	40bc	47bc	59b	39ab

TABLE 20.--The effects of nitrogen nutrition and temperature on the glutamine nitrogen and asparagine nitrogen fractions in the tops of Italian ryegrass grown in a soil-sand mixture in a growth chamber (means of 3 replications).

Fertility Treatment	Glutamine N		Asparagine N	
	Day/Night Temperature			
	21/16 C	32/26 C	21/16 C	32/26 C
	ug N/g DW		ug N/g DW	
+Low Nitrate N	69 a	70a	131a	99a
+High Nitrate N	46a	132a	101a	165a
+Low Ammonium N	24a	100a	123a	161a
+High Ammonium N	379b	320b	347b	321b

DISCUSSION OF RESULTS

A definite interaction between nitrogen source and level and temperature on the growth of the cool-season grasses in both the nutrient solution and soil experiments occurred. However, the interaction was not the same for both types of experiments. In the nutrient solution experiment, growth was always reduced by the high temperature with a greater reduction at the high level of nitrogen. On the other hand, growth was reduced only slightly or not at all at the high temperature and low nitrogen level in the soil experiments. At the high nitrogen level growth was reduced at the high temperature compared to the low temperature although the growth at the high temperature was not less than that at the low nitrogen level.

One would not necessarily expect the same results from nutrient solution and soil experiments. Soil has a buffering capacity for many nutrient ions including ammonium ions due to ion exchange on charged sites. Thus the concentration of ions in the soil solution may be less than in a nutrient solution yet the ions available to the plant for growth from the soil system may be as high or higher. Also there are microbial relations in the soil that do not exist in the nutrient solution. Another very important factor is the loss of nitrogen from the soil

experiments as nitrogen was utilized by the growing plants and leached out in drainage water. The nitrogen level in the soil was probably much lower by harvest time than at the start of the experiments. The low levels of the various nitrogen fractions determined in the soil experiments indicated that levels of available nitrogen had become quite low in the soil experiments. However, the yields and responses of the grasses to nitrogen in the soil experiments indicated that they were not starved for nitrogen during the entire experimental period. In contrast to the nitrogen losses with the soil experiments, the nitrogen levels in the nutrient solution experiment remained constant throughout the experimental period since the solutions were replaced daily.

Ammonium-N and nitrate-N produced different effects on growth. The results from the nutrient solution experiment agreed in part with those of Darrow, Harrison, and Sprague (7, 12, 25), who found nitrate-N to be superior to ammonium-N as a nitrogen source for grasses grown in nutrient solution. Experiment 1 gave results similar to theirs at the high level of nitrogen; however, at the low nitrogen level and low temperature nitrate-N and ammonium-N were equal as nitrogen sources. In Experiment 3, a soil experiment, in which nitrification of ammonium nitrogen was inhibited, ammonium-N was found to be superior to nitrate-N for top growth. However, since these cultures

were heavily watered, it is possible that this result was due to greater leaching losses of nitrate-N than ammonium-N rather than to a specific effect of the ammonium ion.

The content of soluble carbohydrates was somewhat lower at the high temperature for some treatments, but this result was not consistent for all treatments and all experiments. In no case was the soluble carbohydrate exhausted, nor did it appear to be present in concentrations low enough to limit growth.

Nitrogen metabolism was significantly affected by both nitrogen nutrition and temperature. In the nutrient solution experiment there were no significant differences in protein nitrogen content of the tops due to nitrogen nutrition. However, there may have been a tendency for a small increase with ammonium-N compared to nitrate-N and possibly a small increase with the high level of nitrogen. There was also some indication of a decrease in protein nitrogen at the high temperature. Also the soluble amino nitrogen content generally increased at the high temperature and at the high nitrogen level with ammonium-N, but these changes did not occur with nitrate-N. This result may indicate that some sort of regulation of the maximum amount of nitrate-N that can be reduced and assimilated into organic forms occurs, whereas with ammonium-N, assimilation continues as long as ammonium

ions are taken up. Such a regulation has been observed in blue-green algae (Chlorella species) (30,31).

Increases in soluble amino nitrogen such as occurred at the high temperature with ammonium-N were observed by Sullivan and Sprague (28) and Petinov and Molotkovskii (21,22). They attributed these increases to increased protein breakdown without resynthesis. However, such increases might also occur if protein synthesis were blocked or slowed down. In the nitrate-N treatments increases in soluble amino nitrogen did not occur, which may indicate that blockage of protein synthesis was more important in the high temperature effects found than was protein hydrolysis.

The results of the soil experiments also indicated that a blockage or slow down of protein synthesis was more likely to be involved than an increase in protein hydrolysis. In Experiment 2 when no nitrogen was added, the protein nitrogen content was lower at the low temperature than at the high temperature. When nitrogen was added, no differences due to temperature occurred. Following the first harvest, as growth continued at the low temperature, protein nitrogen decreased for all treatments. At the high temeprature, as a small amount of regrowth occurred in the tops, protein nitrogen dropped. However, in the roots where no new growth occurred, protein nitrogen remained the same. Thus, the lower protein nitrogen at the low temperature when no nitrogen was added

and the decreases in protein nitrogen between harvests at the low temperature seem to indicate that as growth continued and as available nitrogen became low, as probably occurred in the soil experiments, protein was broken down in the older portions of the plants and resynthesized for new growth. The decreases in protein content observed were not likely to be simply dilutions with growth since new growth is generally high in protein content. On the other hand, at the high temperature little or no growth was occurring, possibly because of a lack of protein synthesis, so no breakdown and resynthesis of protein occurred.

If protein synthesis were blocked one would also expect some increase in soluble amino nitrogen as these precursors to protein built up. If available nitrogen were low, the build up would not be expected to be as great. On the other hand, if a rapid hydrolysis of protein without resynthesis were occurring, one would expect the soluble amino nitrogen to remain high even if available nitrogen dropped. The changes in soluble amino nitrogen observed in the soil experiments followed the former pattern. The soluble amino nitrogen content reached quite low levels especially in Experiment 3. It also decreased between harvests in Experiment 2. Although the soluble amino nitrogen content remained higher at the high temperature compared to the low temperature, the proportion of soluble amino nitrogen to protein

nitrogen actually decreased between harvests at the high temperature, a result that would not be expected if a general breakdown of protein were occurring.

Increases in free ammonium and amide nitrogen are also generally associated with protein breakdown, but these fractions remained low at the high temperature, although they did respond to increased nitrogen levels.

It is possible that some injury could occur from an accumulation of some particular soluble nitrogen fraction, e.g. free ammonia (11, 14). No particular soluble nitrogen compound accumulated to high levels in the soil experiments. In the nutrient solution experiment the soluble nitrogen fraction was much higher, but the constituents of this fraction were not determined. Thus, it is possible that a particular toxic compound might accumulate at higher nitrogen levels. This could also happen at higher temperature conditions such as used by Petinov and Molotkovskii (21, 22).

The interactions of nitrogen source and level and temperature on growth and on the chemical composition of cool-season grasses indicate that these variables must be carefully controlled in future studies of the biochemical basis for high temperature growth reduction.

CONCLUSIONS

1. An interaction between nitrogen nutrition and temperature on the growth of cool-season grasses occurred. In a nutrient solution experiment, where the nitrogen levels remained constant throughout the experimental period, growth was always reduced by the high temperature, and the reduction was greater at high levels of nitrogen and with ammonium-N. At the low temperature an increase in level of nitrate-N increased growth while an increase in level of ammonium-N decreased growth. In a soil experiment in which nitrification of added ammonium-N was not prevented, additions of either ammonium or nitrate-N caused an increase in growth of tops at the low temperature but not at the high temperature. In a second soil experiment in which nitrification of added ammonium-N was prevented, ammonium-N was superior to nitrate-N for top growth. An increase in growth for the high level of ammonium-N but not for the high level of nitrate-N occurred at the low temperature. No response to source or level of nitrogen occurred at the high temperature.

2. Changes in soluble carbohydrate content did not appear to be the causal factor of growth reduction at the high temperature nor for the interactions found. In no case

was soluble carbohydrate exhausted, nor present in low enough concentrations to limit growth.

3. In the nutrient solution experiment protein nitrogen content appeared to be slightly increased by ammonium-N compared to nitrate-N and slightly decreased by high temperature. The soluble amino nitrogen content generally was higher with ammonium-N than nitrate-N and increased at the high temperature with ammonium-N but not with nitrate-N.

4. In the first soil experiment protein nitrogen content was generally lower at the low temperature than at the high temperature with no added nitrogen. When nitrogen was added, no differences due to temperature occurred at the first harvest. Between harvests protein nitrogen content decreased where growth continued but remained constant in the roots at the high temperature where no growth occurred. The soluble amino nitrogen content was generally higher with nitrogen additions and at the high temperature. The level of available nitrogen appeared to exert a greater effect than did temperature. Between harvests the soluble amino nitrogen content decreased at both temperatures. In the second soil experiment where free ammonium, nitrate, glutamine, and asparagine nitrogen were determined, these fractions all were quite low and generally responded to an increase in ammonium nitrogen level but not to temperature.

5. The effects of nitrogen nutrition and temperature on nitrogen metabolism appeared more likely to involve a blockage or slow down of protein synthesis rather than an increase in protein breakdown. An accumulation of some toxic nitrogen compound at high nitrogen levels could also be involved.

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