TRENDS IN NITRATE REDUCTION AND NITROGEN FRACTIONS IN CORN PLANTS DURING MOISTURE STRESS

by

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TABLE OF CONTENTS

INTRODUCTION

Almost every year drought is a problem somewhere in the Great Plains of the United States. Drought reduces the quality and quantity of plant material produced and is a major factor in preventing stabilization of agriculture in the region.

Nitrate absorbed by the plant must be reduced to ammonia or hydroxylamine before it can be utilized by the plant for amino acid and protein synthesis. The first step in this reduction process is the conversion of nitrate to nitrite by the enzyme nitrate reductase. With available nitrate nitrogen, reduced activity of this enzyme results in nitrate accumulation.

In some cases nitrate has accumulated in sufficient amounts to be toxic to livestock. Reduced nitrate reductase activity has been attributed to decreased light, low moisture, low fertility, genotype differences, and possibly other factors. Molybdenum has been reported to be a metal constituent of nitrate reductase in certain plants. These findings suggest that moisture stress may influence nitrate reduction by limiting molybdenum uptake or availability.

Decreased nitrate reduction in plants under moisture stress may influence the quantity and/or quality of other nitrogen fractions and of total nitrogen in the plant. Little information of this type is available, however. Also, little is known of the influence of moisture stress on nitrogen metabolism in different parts of the plant or in plants of different ages or sizes. Therefore, experiments were conducted with the following objectives: (1) To determine the relative changes in nitrate reductase activity in various parts of corn plants at different ages and under varying

degrees of moisture stress, (2) to determine the contents of nitrate, various nitrogen fractions, and total nitrogen of various parts of corn at different ages under varying degrees of moisture stress, (3) to determine the relative amounts of molybdenum in young corn plants exposed to varying degrees of moisture stress, and (4) to compare the influence of moisture stress on these factors.

REVIEW OF LITERATURE

Nitrate Reductase

The nitrate-reducing properties of plant sap were not clearly understood for some time although as early as 1903, Pozzi-Escot (19) noted that expressed plant juice could reduce nitrate to nitrite. Anderson (1) in 1924, demonstrated the ability of sap expressed from 23 different species to reduce nitrate to nitrite, but attributed this reduction to a reducing substance in the sap rather than to an enzyme. In the same year, Eckerson (6) showed that nitrite was formed when expressed plant sap was incubated with nitrate and glucose and concluded, without proof, that reduction was brought about by an enzymatic system.

Proof that nitrate was reduced to nitrite by an enzymatic process came in 1952, when Evans and Nason (7) reported purification of a nitrate reductase enzyme from soybean leaves. The enzyme was sensitive to concentrations of nitrate, with 2×10^{-2} M potassium nitrate causing saturation. Reductase activity in the leaves was increased when flavine adenine dinucleotide (FAD) was added but was very slightly affected when flavine mononucleotide (FMN) was added, indicating that the natural prosthetic group of the enzyme was FAD. Nitrate reductase from the majority of sources

studied has shown similar properties. Spencer (23) indicated the enzyme in embryos of germinating wheat was in the soluble.cytoplasmic fraction of the cell, had an optimum pH level for maximum activity, and was very sensitive to heat and storage since 38% of the activity was lost at -15°C. for a week.

Hageman and Flesher (10) reported that in corn leaves, both light and nitrate are necessary for maximum nitrate reductase activity. In cauliflower (3) and in corn (10), prolonged darkness depresses the nitrate reductase activity, which is restored rapidly upon exposure to light. In agreement with this relationship, a significant positive correlation was found between enzyme activity and water soluble protein and a negative correlation with nitrate accumulation.

Molybdenum

Most of the information available concerning molybdenum in plants is related to its role in nitrate reductase activity. Inhibition of nitrate reductase from Neurospora (14) and soybean leaves (7) by potassium cyanide, sodium azide, thiourea, and potassium ethyl xanthate indicated a heavy metal requirement. Later, it was found that molybdenum is the metal associated with the enzyme from these two sources (15, 4). In 1939, Arnon and Stout of the University of California showed the necessity of molybdenum for tomatoes. Other evidence indicating that molybdenum is the metal constituent of nitrate reductase from different sources was as follows:

(1) Cauliflower plants (3) as well as Neurospora and Aspergillus (17, 18) deficient in molybdenum possessed lower nitrate reductase activity than those grown with a normal supply of molybdenum and the activity was increased in the deficient organisms after they were supplied with molybdenum.

(2) Removal of the metal constituent of the enzyme from Neurospora (15, 17) and soybean leaves (16) or Aspergillus (17) by dialysis resulted in loss of activity which was restored by addition of molybdenum trioxide or sodium molybdate while many other metals did not restore the lost activity.

(3) There was a direct relationship between the specific activity and the molybdenum content per milligram of protein of various enzyme preparations from soybean leaves (16), Neurospora (15, 17) and Aspergillus (17). Moreover there was no consistent relationship between specific activity and content of a number of other metals in the soybean leaves enzyme (16).

Nitrogen

The nitrogen of proteins may be translocated from older tissues to the younger and more actively growing points, where it is reutilized in protein synthesis. Gates (9) found that translocation of nitrogen and phosphorus from the laminae to the stem occurred in tomatoes as a response to wilting.

Accumulation of certain nitrogen compounds also occurs in plants during moisture stress. As early as 1895 Mayo (13) reported losses of cattle that had eaten corn fodder containing up to 25% potassium nitrate and in which crystals of potassium nitrate could be seen. Wadleigh and Ayers (25) reported that increasing moisture stress in beans tended to cause an increase in the percentage of nitrate nitrogen and soluble organic nitrogen in the plant and also an increase in the percentage of protein in the leaves. Flynn et al. (8) observed that nitrate uptake of corn under drought exceeded the capacity of the forage to reduce it and

nitrate accumulated primarily in the stalk and persisted at toxic levels in the plants.

Results on the effect of moisture stress on total nitrogen content have been most contradictory. It is generally agreed, however, that protein content increases in years of limited moisture.

Experiments separating total nitrogen into water insoluble nitrogen, water soluble protein nitrogen, and water soluble non-protein nitrogen under moisture stress conditions were not found in reviewing the literature. However, Yarosh (cited by Vaadia et al. (24)) reported the ratio of non-protein to protein nitrogen was between .14 to .24 in unirrigated cotton and .11 to .19 in irrigated cotton. He attributed this change to decreased synthesis and increased hydrolysis.

MATERIALS AND METHODS

Double cross K-1859 corn produced on the Roepke Seed Farm, Manhattan, Kansas in 1961 was used in all experiments.

Plants were grown in small growth chambers having a temperature of 21-22°C. at plant height, a 16 hour photoperiod (4 a.m. to 8 p.m.), and a light intensity of approximately 2,000 foot candles. Two ages of corn were analyzed: (I) the 4-leaf stage when plants were approximately 6 to 8 inches tall and (II) the 7-leaf stage when plants were approximately 28 inches tall. All plants were sampled at 9 a.m.

Age I experiments were conducted with 6-inch plastic pots, 12 plants per pot. Vermiculite was used as the growth medium and was maintained at field capacity with double strength Hoagland No. 1 nutrient solution.

Age II plants were grown in 7-inch cylindrical glazed crocks filled with vermiculite, one plant per crock. Field capacity was maintained with 4x Hoagland No. 1 nutrient solution. In preliminary trials using double strength Hoagland No. 1 nutrient solution, iron deficiency symptoms were noted the third to fourth week after planting. When the nutrient solution was raised to 4x strength, no visual deficiency symptoms appeared, so this concentration was used throughout the Age II experiments.

At the desired age, the vermiculite was saturated with nutrient solution, allowed to drain to field capacity, and plants were immediately placed under stress conditions.

Moisture stress was imposed by raising the temperature to 37-38°C. under decreasing humidity created by a small dehumidifier set on continuous run. The relative humidity gradually decreased from 100% to approximately 40% when the last samples were taken.

Plants were sampled for analyses at intervals until permanent wilting. Age I plants were cut off at the vermiculite line and the entire tissue utilized. Age II plants were separated into leaf blades, leaf sheaths, leaf rolls, and stems. It would be well to note that due to limited light available to lower leaves and the size of the growth chambers available, Age II plants sometimes varied greatly in size and therefore greatly in results. Also, the individual plant compositions were invariably different, and exact separation of a plant into its component parts was difficult.

Moisture Stress and Per Cent Moisture Determinations

The water status of Age I corn was determined by modification of the relative turgidity test as outlined by May and Milthorpe (12). Three plants

were weighed immediately after harvesting, allowed to regain full turgidity by placing in a saturated atmosphere for 6 hours and then reweighed. The per cent relative turgidity was expressed as a ratio of water actually present to that held at full turgidity. Preliminary tests using the specific conductivity method outlined by Dexter, Tottingham, and Graber (5) to determine hardiness were inconsistent and unreliable. Moisture percentage was determined by weighing tissue before and after drying for 22-24 hours at 70°C.

Nitrate Reductase Determinations

Extraction was accomplished by modification of the refined procedure used by Hageman and Flesher (10). The nitrate reductase assay method, as well as the nitrate method used, is based on a colorimetric determination of the nitrite produced. In these reactions, nitrite reacts with sulfanilic acid to produce a diazonium salt which then is coupled with 1-naphthylamine to form a red color. Duplicate samples of material were blended in media in a ratio of 1:4 by weight. The blending media was composed of .1 M tris hydroxymethyl-aminomethane, 0.01 M cysteine, and 0.003 M ethylenediamine-tetraacetic acid adjusted to pH 7.2 with dilute hydrochloric acid to give the final homogenate a pH of 7.0. Blending was accomplished by a Servall omnimixer at 16,000 rpm for one minute, with the blending cup immersed in an ice bath. The cup was then removed, any adhering tissue pushed down into the cup, and the mixture was reblended for another minute. Unmacerated tissue often adhered to the side of the cup if blended continuously and preliminary trials showed continuous blending often destroyed some of the enzyme. The two 1-minute interval method used minimized tissue

adhering to the side of the cup and enzyme destruction by heat. The extract was filtered through a fine mesh sieve and centrifuged at 2°C. for 15 minutes at 20,000 x G. The supernatant was used for analysis. The extracts were kept at 2-3°C. throughout the entire analysis.

Nitrate reductase activity was measured by modification of the Evans and Nason method as reported by Hageman and Flesher (10). The assay mixtures, which were made the day before sampling, contained 1 ml. of 0.1 M potassium phosphate buffer (pH 7.2), and 0.2 ml. of .1 M potassium nitrate.

The assay was initiated by adding first 0.3 ml. of enzyme extract and immediately 0.5 ml. of 1.36×10^{-3} M of diphosphopyridine nucleotide (DPNH)*, which was prepared just prior to using. The resulting mixture was then incubated at 27°C. for 30 minutes after which the reaction was stopped by adding 1.0 ml. of 1% (w/v) sulfanilic acid in 1.5 N hydrochloric acid. Then, 1.0 ml. of .02% (w/v) ethylene diamine dihydrochloride was added, the contents of the tube inverted, and the color was allowed to develop for 10 minutes. The per cent transmittance was then read directly, or by a 1:9 dilution with redistilled water, on a Bausch and Lomb Spectronic 20 colorimeter at a wavelength of 540 millimicrons.

Preliminary trials showed no difference in a 30 or 45 minute incubation period. Sulfanilic acid, which is more stable in solution but requires a longer period for full color development was substituted for sulfanilamide.

Standard curves for nitrate reductase activity were made by substituting known concentrations of sodium nitrite for the enzyme extract. A straight line reference was obtained by plotting percentage transmittance against concentration on semilog paper.

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* This compound is synonymous to reduced nicotine adenine dinucleotide (NAD-2H).

Nitrate and Nitrite Determinations

Extraction of nitrate was the same as for nitrate reductase, except that plant material was blended in water at the ratio of 1:5 by weight for the Age I corn experiments. Nitrate concentrations were so high in the Age II corn that a plant material to water ratio of 1:100 or 1:200 was required in the later stages of moisture stress. The total or a portion of the filtrate was then centrifuged at 6,000 x G for 15 minutes.

Determinations of nitrate were made by further modification of the Nelson, Kurtz, and Bray method as reported by Wooley, Hicks, and Hageman (26). One ml. of centrifuged extract was added to 9 ml. of 20% acetic acid containing 0.2 ppm copper as copper sulfate. Immediately thereafter 0.4 grams reducing powder was added. The reducing powder consisted of 100 grams barium sulfate, 75 grams citric acid, 10 grams manganous sulfate dihydrate, 4 grams sulfanilic acid, 2 grams of 1-naphthylamine, and 2 grams of powdered zinc. The test tubes were then stoppered, shaken for 15 seconds and allowed to stand undisturbed for three minutes. After repeating this procedure three times the mixture was centrifuged at 6,000 x G for 5 minutes. The per cent transmittance of the supernatant was then read on a Bausch and Lomb Spectronic 20 colorimeter at a wavelength of 520 millimicrons directly, or was diluted 1:9 with redistilled water when necessary. Nitrate standard curves were made by substituting known concentrations of calcium nitrate for the extract. The transmission percentages were plotted in the same manner as for nitrate reductase.

Intensity of the color was decreased when limiting or excessive amounts of zinc were used, therefore the reducing powder was weighed before adding

it to a sample. Nitrate determined by this procedure includes the nitrite present in the material. However, nitrite levels were so low in comparison to nitrate levels, that no corrections were made.

Nitrite was determined by the above procedure omitting manganous sulfate and powdered zinc from the reducing powder. Without these components nitrate was not reduced to nitrite and only the nitrite present in the original extracts was measured. However, concentrations were so low as to indicate no accumulation of nitrite.

Molybdenum Determinations

Plants grown for molybdenum determination were harvested, weighed, and dried for at least 24 hours. Molybdenum content was determined by further modification of the method described by Jackson (11). The plant tissue was ground finely in a micro Wiley mill and at least 15 grams were weighed, placed in a porcelain crucible, and ashed at 500°C. for 24 hours. The ash was placed in a platinum crucible and fused in approximately 4 grams of anhydrous sodium carbonate to effect solution of molybdate as the sodium salt and to separate most of the interfering substances as insolubles in the subsequent water extract. The sodium carbonate cake was transferred to about 200 ml. of water containing 2% ethanol and heated for 2 to 3 hours on a warm hot plate to hasten disintegration. The mixture was then centrifuged at room temperature for 10 minutes at 27,000 x G, and the clear supernatant was poured off and acidified to remove the silica, using 2 ml. of concentrated hydrochloric acid per gram sodium carbonate contained. The solution was evaporated to dryness, 15 ml. of 3 N hydrochloric acid added to the residue and briefly warmed. The mixture was centrifuged at room

temperature for 10 minutes at 27,000 x G. to remove the precipitate. The clear supernatant was transferred to a 25 ml. volumetric flask, 1.5 ml. of 10% potassium thiocyanate and 8 ml. of acetone were added and the solution was made to volume with redistilled water and mixed. The solution was then digested in a 60-70°C. water bath for 2 to 3 hours to remove the red color of $Fe(SCN)_3$, cooled to room temperature, made to volume with redistilled water, mixed, and centrifuged again for 10 minutes at 27,000 x G to remove turbidity. Percentage transmittance was read directly on the supernatant using a Bausch and Lomb Spectronic 20 colorimeter at a wavelength of 470 millimicrons. Single determinations were made in each of two experiments.

Molybdenum concentrations were determined from a standard curve made by substituting known concentrations of H_2MoO_4 for the plant samples.

Nitrogen Fraction Determinations

Non-soluble (water insoluble) nitrogen was determined by further modification of the method used by Siminovitch and Briggs (21). A known weight of fresh tissue was blended in approximately 100 ml. of water in Servall omnimixer at 16,000 rpm for one minute, with the cup immersed in an ice bath. The cup was then removed, any adhering tissue pushed down into the cup, and the mixture reblended for another 30 seconds. The mixture was filtered through a Whatman No. 4 filter paper, the cup and cutting assembly were rinsed with distilled water several times, and the rinsings were used to wash the macerated plant tissue retained by the filter.

Water soluble protein nitrogen and water soluble non-protein nitrogen were determined from the combined filtrate and washings. The filtrate was

transferred to a beaker, and the pH was adjusted to approximately 4.0 with glacial acetic acid. The solution was heated to near boiling to coagulate the protein, and cooled rapidly. The cooled solution was filtered through a Whatman No. 2 filter paper and the precipitate and beaker were rinsed several times with distilled water. The precipitate constituted the water soluble protein nitrogen and the filtrate combined with the washings constituted the water soluble non-protein nitrogen. Duplicate determinations were made in each of two experiments.

The nitrogen content of each of the 3 fractions was determined by the Gunning modification of the Kjeldahl method (2) using boric acid in the receiving flask (20). Total nitrogen was computed as a summation of the values obtained for each of the three fractions.

Statistical analyses were made as outlined by Snedecor (22).

RESULTS AND DISCUSSION

Moisture Stress

Changes in water content and relative turgidity for Age I plants are shown in Fig. 1 and individual data are shown in appendix table 1. Moisture percentage data for Age II plants are given in Fig. 9 and in appendix table 4.

Water content of Age I corn decreased slowly, but consistently after the first day of stress as would be expected. Per cent turgidity likewise remained relatively constant during the first two days of exposure to stress. Thereafter a constant and sharp decrease occurred, indicating that diffusion pressure deficit (DPD) or moisture stress in the plant began after the second day and increased with continued exposure.

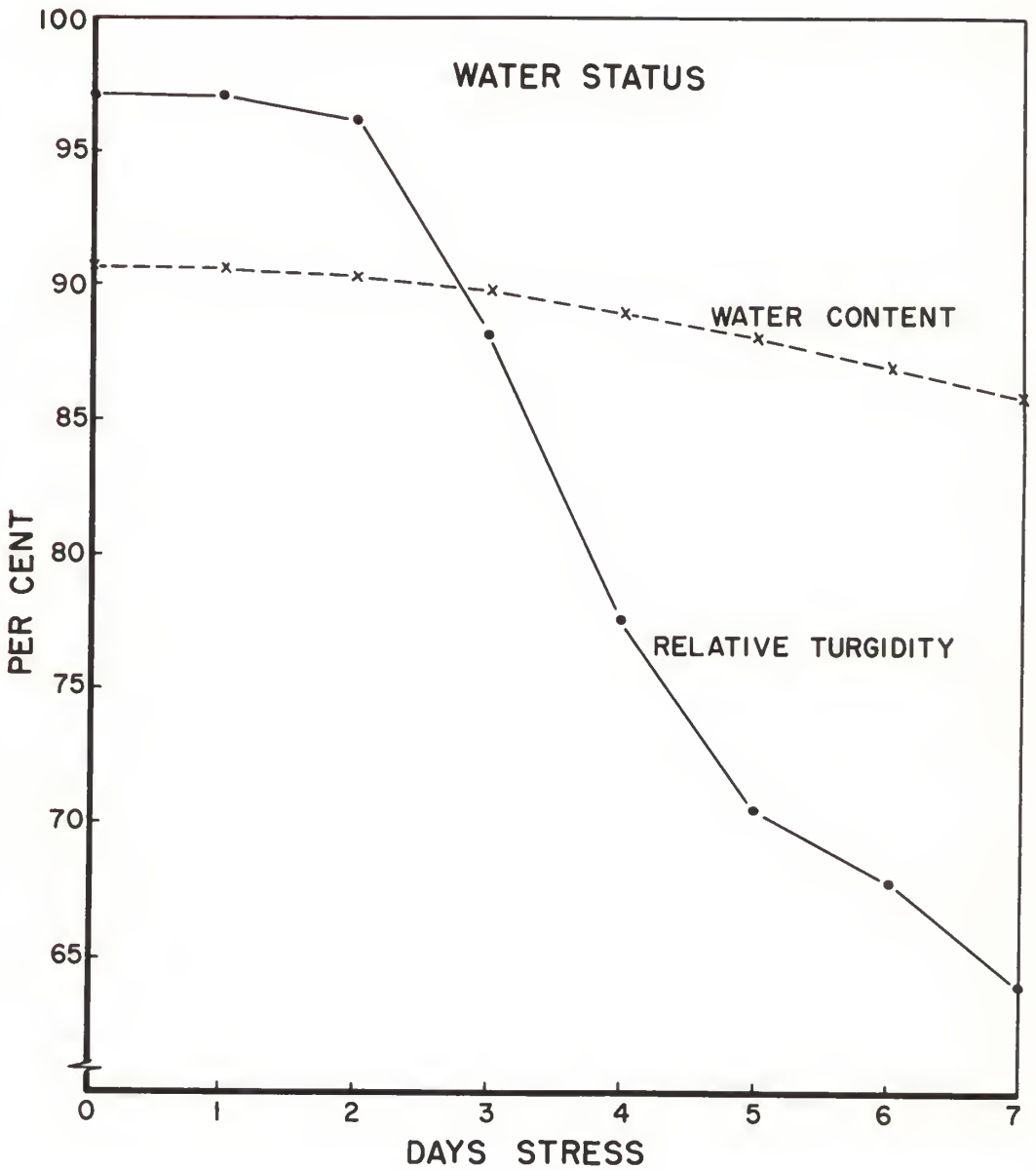


Fig. 1. Moisture percentage and relative turgidity in Age I plants.

Since both relative turgidity and water content decreased steadily during exposure to stress, either method might be used as an indicator of degree of stress. Relative turgidity decreased more rapidly with increased stress, however, and appeared to be much more sensitive to changes in water status within the plant than did percentage water.

Because of limited growth chamber space and resulting lack of experimental material, only percentage moisture determinations were made with Age II plants. General agreement between percentage moisture curves for Age I and Age II plants, however, indicated that the same relative pattern of increase in stress was obtained with whole plants of each age. Age II plants began to visibly wilt after the first day of stress. Experiments were concluded after five days of stress shortly before apparent permanent wilting.

In Age II experiments the water content of the sheath and stem remained relatively constant or decreased only slightly throughout the stress period. Both the blade and the leaf roll increased in water content the first day of stress, but consistently decreased at approximately the same rate thereafter; however, the roll was always higher in water content.

Differences in rate of decrease in water content among plant parts likely reflect the greater surface area and transpiration characteristics of the leaf blade and roll in contrast to the stem and sheath. Most of the roll was protected by leaf blade and sheath and was desiccated less than the more exposed blade.

Nitrate Reductase Activity

Effects of stress on nitrate reductase activity are illustrated in Figs. 2 and 4. Individual data are reported in appendix tables 1, 3, and 7.

In Age I corn, effect of moisture stress on nitrate reductase activity was immediate. Activity decreased significantly during each of the first four days of stress. Afterward it remained relatively constant, but at an activity level of less than one-twelfth of the non-stress level (per gram fresh basis). Decreased activity was noted before moisture content was decreased by stress (Fig. 1). This seems to indicate that enzyme activity is influenced by changes in moisture stress too small to be measured by relative turgidity, or by factor(s) other than moisture.

Activity in all parts of Age II corn decreased significantly the first day of stress. Enzyme activity in the stem, sheath, and roll after the first day of stress remained relatively nil. In some cases concentrations of nitrite were so low as to make exact determinations difficult. The level of activity in the blade was highest of any other component part. After a significant decrease the first day of stress, activity in the blade remained almost constant until the fourth and fifth days when a significant decrease occurred again. Most of the nitrate reduction occurred in the blade and the process was least affected by moisture stress.

It should be noted that decrease in nitrate reductase activity in Age II plants was not necessarily associated with percentage moisture. In contrast, moisture content in the leaf sheath and stem changed very little during the experiment while enzyme activity decreased rapidly. In the leaf roll and blade, decreased activity preceded decreased moisture content.

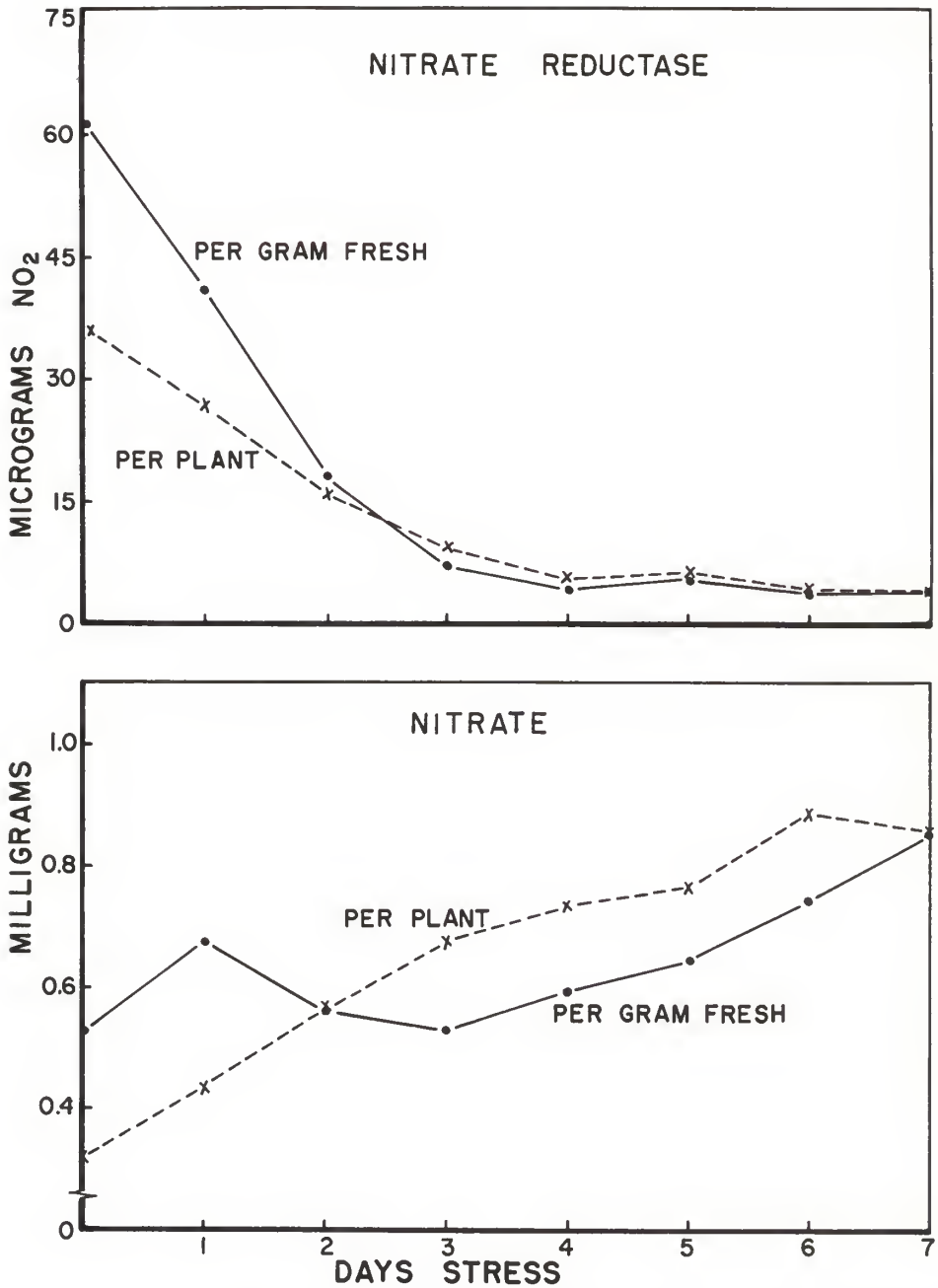


Fig. 2. (Upper) Nitrate reductase activity in Age I plants.

Fig. 3. (Lower) Nitrate nitrogen content in Age I plants.

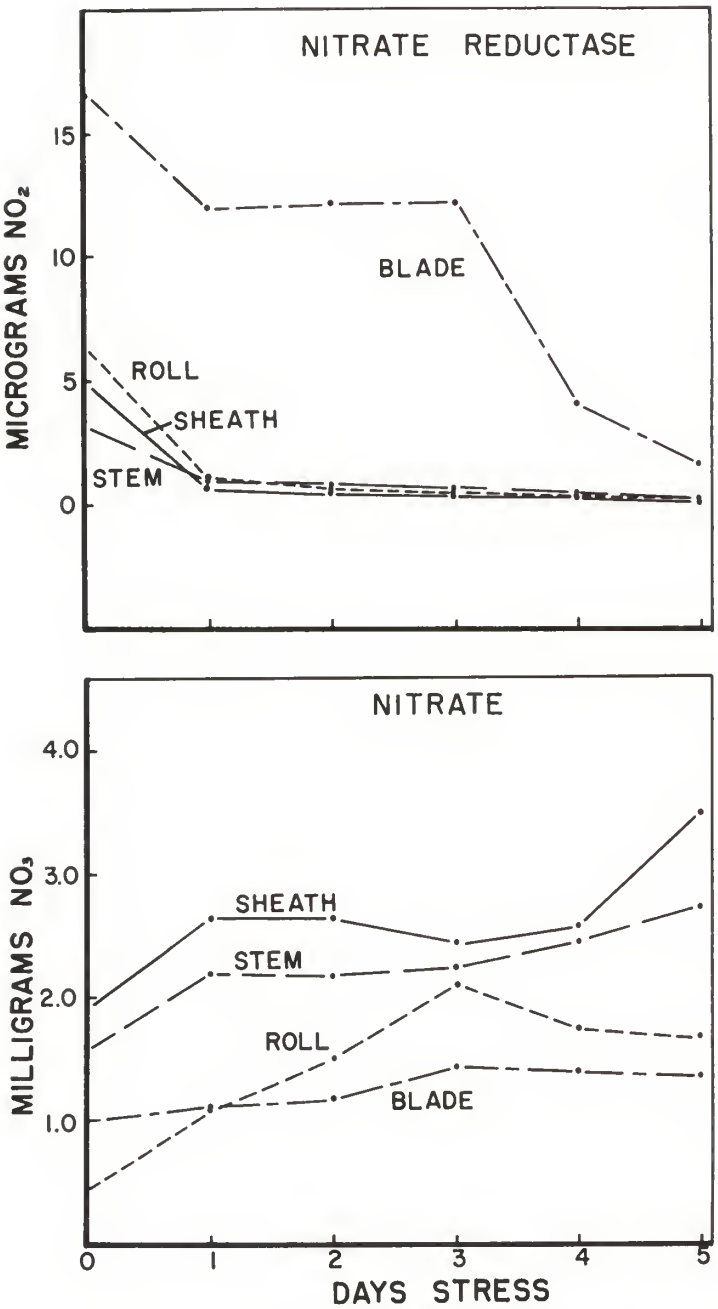


Fig. 4. (Upper) Nitrate reductase activity per gram fresh weight in different parts of Age II plants.

Fig. 5. (Lower) Nitrate nitrogen content per gram fresh weight in different parts of Age II plants.

Nitrate

Results of nitrate determinations are shown in Figs. 3 and 5 and in appendix tables 1, 3 and 6. Nitrate content per gram fresh weight in Age I plants increased the first day of stress and decreased during the second and third days. Afterward nitrate concentration increased steadily. Quantities of nitrate per plant increased consistently during the first six days of the stress period.

Increased nitrate per plant indicated continued absorption by plants during the stress period. Results of nitrate determinations reflect net accumulation over reduction; thus, actual uptake of nitrate probably was greater than indicated. During the first two days of exposure to stress conditions, relative turgidity of plants remained at a high level (Fig. 1), indicating that conditions existed for rapid growth and maximum nitrate absorption. Nitrate reductase activity, however, was decreasing. This resulted in an accumulation, i.e., higher concentration, of nitrate. During the second day of exposure, nitrate reductase activity decreased further. Concentration of nitrate decreased, however, because internal moisture balance still permitted a large net increase in fresh weight of plants. This pattern continued at a reduced rate from the second to the third day as relative turgidity decreased more rapidly. After the third day of exposure, nitrate reductase activity had practically ceased, internal moisture stress became severe, and growth rate apparently decreased. Thus, absorbed nitrate was not reduced and accumulated both in concentration and per plant.

In Age II plants (Fig. 5) concentrations of nitrate were highest in the sheath, but only slightly greater than in the stem. Nitrate concentration in the leaf roll increased rapidly during the first three days of stress, but

decreased slightly thereafter and never was as high as in the stem or sheath. In the leaf blade, nitrate content remained almost constant, although a very slight increase was noted from the second to third day of stress.

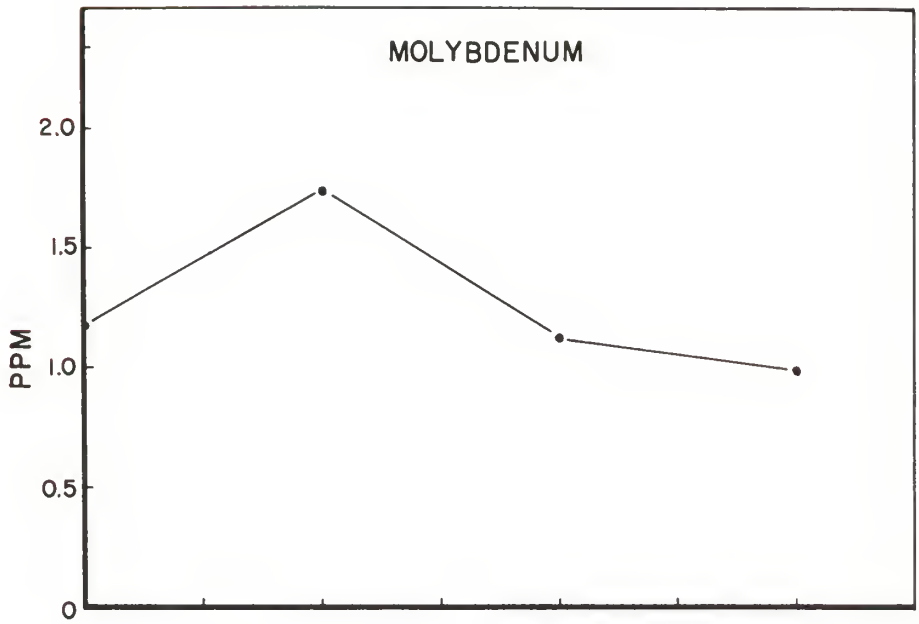
In the stem and sheath, nitrate reductase activity was low initially and decreased very rapidly to an extremely low rate with brief stress exposure. Thus, nitrate accumulated in these plant parts during the entire experiment.

Nitrate concentration continued to increase in the leaf roll during the first three days of stress. Also, moisture content, while decreasing, was higher than in the leaf blade where nitrate concentration did not increase. Thus, continued fresh weight accumulation in the leaf roll should have resulted in decreased rather than increased concentration. Since the leaf roll represents the plant part with the highest rate of metabolic activity, the possibility exists that nitrate was translocated from leaf blades to the leaf roll and/or from the soil directly to the roll with less received by blades as moisture stress increased. Low rates of nitrate reductase activity in the roll, however, resulted in accumulation of nitrate.

Translocation of nitrate from leaf blade to roll also may explain the constant nitrate concentration in the blade at a time when nitrate reductase activity was decreasing. Activity of the enzyme was greater than in other plant parts and probably was adequate to reduce the smaller quantities of nitrate.

Total Nitrogen

Trends in total nitrogen in Age I corn plants are shown in Fig. 7 and results may be found in tables 1 and 3 of the appendix.



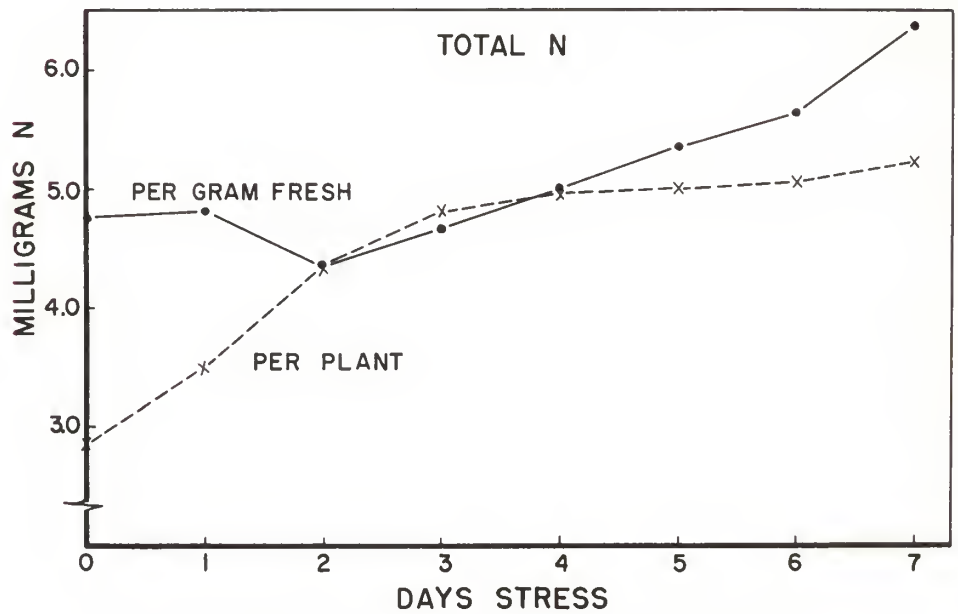


Fig. 6. (Upper) Molybdenum content per gram dry weight in Age I plants.

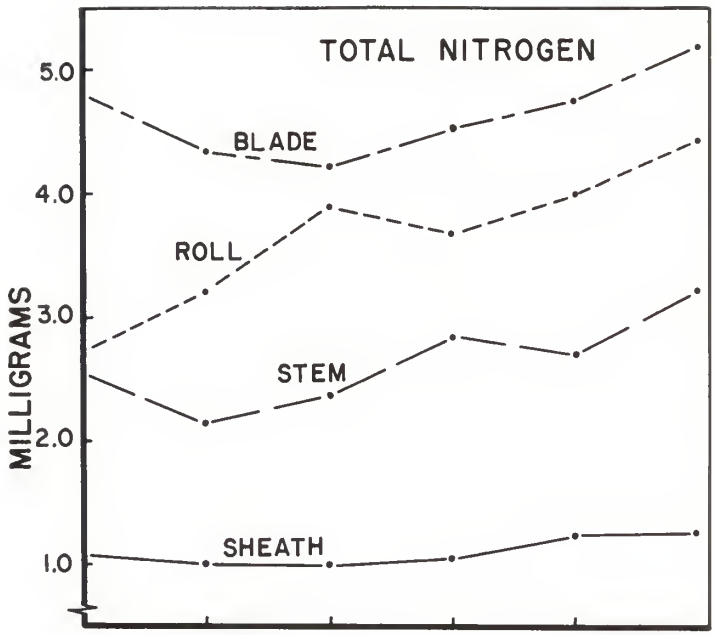
Fig. 7. (Lower) Total nitrogen content in Age I plants.

Total nitrogen per plant in Age I corn increased significantly during the first three days of stress but did not increase thereafter. On a fresh weight basis total nitrogen remained unchanged during the first day of stress and decreased significantly during the next day. After the second day total nitrogen concentration increased significantly throughout the sampling period. Apparently total nitrogen per plant increased to a point at which decreased nitrate reductase activity reduced reduction of nitrate, thus preventing further increase in non-nitrate nitrogen. Concentration of total nitrogen, however, continued to increase as water content decreased.

Trends in concentration of total nitrogen in Age II corn are shown in Fig. 8 and results are listed in table 5 of the appendix.

The low nitrogen content of the sheath remained relatively unchanged throughout the sampling period. Total nitrogen concentrations in the blade, roll, and stem generally increased with induced drought after the second day of stress.

The plant parts in which total nitrogen concentration was highest were lowest in nitrate and generally highest in nitrate reductase activity. Thus, the low level of total nitrogen in the sheath can be associated with high nitrate accumulation and low nitrate reductase activity. Since little nitrate is measured in the Kjeldahl procedure for nitrogen, only small amounts of total nitrogen would have been recovered. In the stem, sheath, and blade increasingly greater nitrate reductase activities resulted in less nitrate accumulation and more total nitrogen as measured by the Kjeldahl procedure.



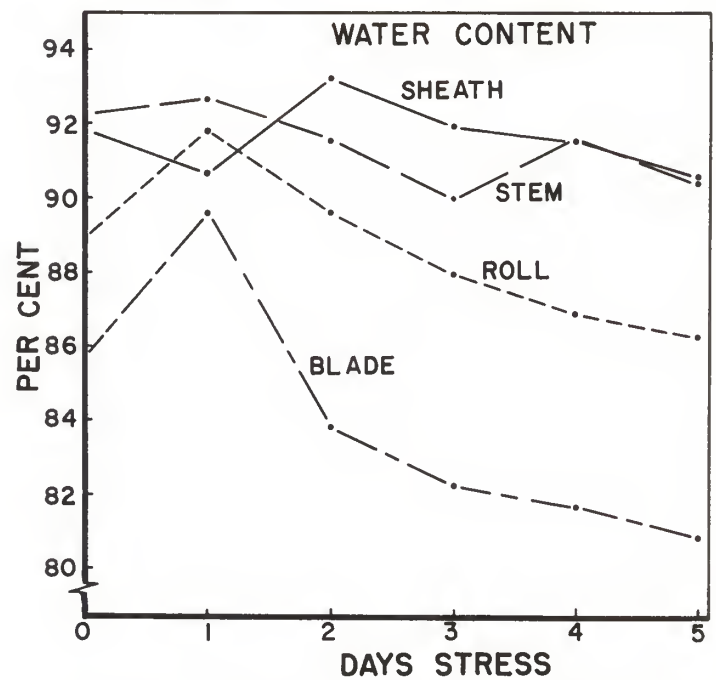


Fig. 8. (Upper) Total nitrogen content per gram fresh weight in different parts of Age II plants.

Fig. 9. (Lower) Moisture per cent in different parts of Age II plants.

Nitrogen Fractions and Per Cent Contribution

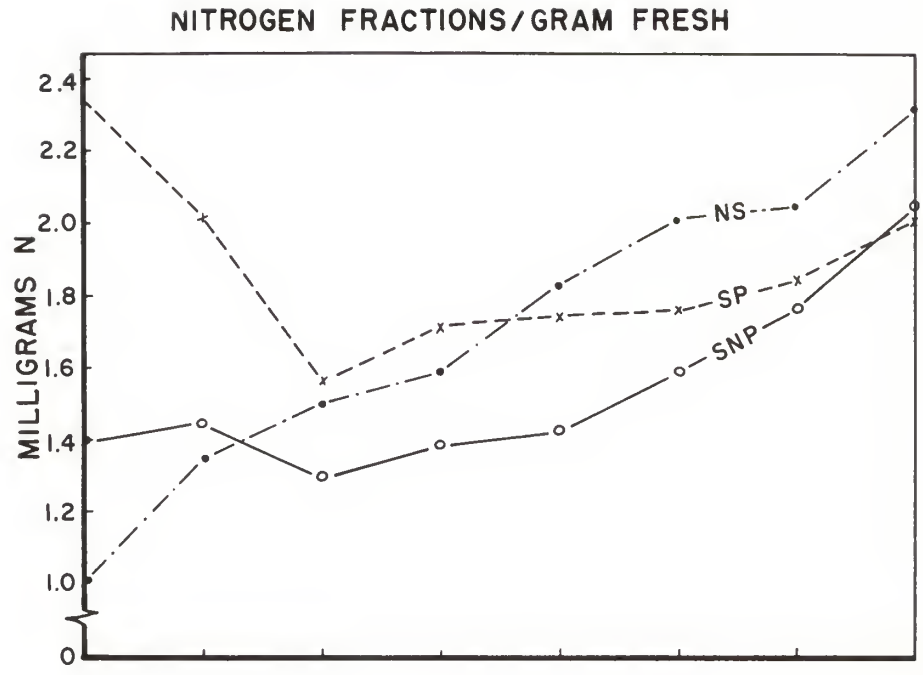
Nitrogen fraction trends in Age I plants are shown in Figs. 10 to 13 and results may be found in tables 2 and 3 of the appendix.

Age I non-soluble nitrogen per gram fresh weight consistently increased throughout the seven-day stress period. However, on a plant basis non-soluble nitrogen increased only during the first four days of stress and remained relatively constant thereafter.

Age I soluble protein nitrogen on a fresh weight basis decreased significantly the first two days of stress. Afterward a slight non-significant but general increase occurred, probably reflecting decreased water content. On a plant basis, no real difference was found in the amount of soluble protein nitrogen during the stress period although a slight increase was noted during the first three days.

Soluble non-protein nitrogen per gram fresh weight remained approximately the same for the first four days of stress after which there was a general increase. On a plant basis, soluble non-protein nitrogen significantly increased during the first two days but thereafter increased only slightly.

As noted in Fig. 7, total reduced nitrogen per plant increased during the first four days of moisture stress. Generally quantities of each nitrogen fraction per plant also increased during earlier stages of stress and, except for an increase in soluble non-protein, remained relatively constant as stress became more severe. Increased concentrations of each fraction on a fresh basis during later stress periods (Fig. 10), therefore, apparently primarily reflect decreasing water content of the plant tissue.



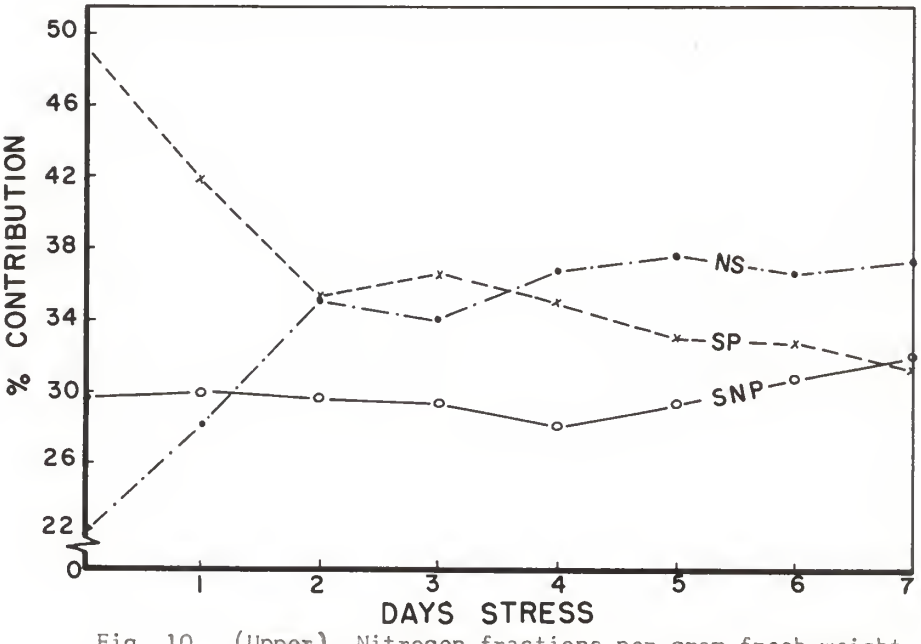


Fig. 10. (Upper) Nitrogen fractions per gram fresh weight in Age I plants.

Fig. 11. (Lower) Contribution of nitrogen fractions to total nitrogen in Age I plants.

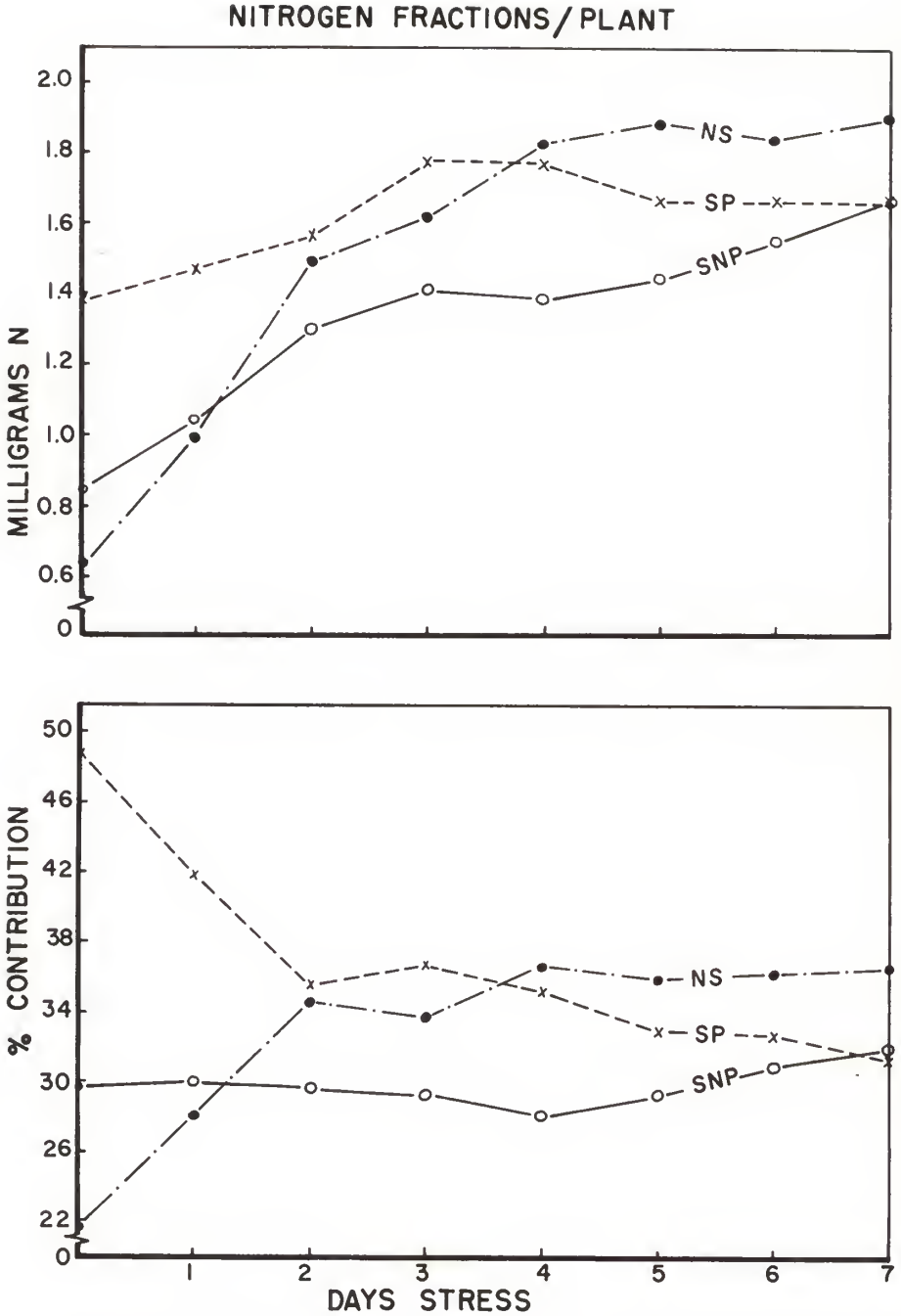


Fig. 12. (Upper) Nitrogen fractions per plant in Age I plants.

Fig. 13. (Lower) Contribution of nitrogen fractions to total nitrogen in Age I plants.

At earlier stages when total nitrogen per plant was increasing, moisture stress apparently influenced greatly the relative distribution of this nitrogen among nitrogen fractions. Soluble non-protein concentration changed little throughout the stress period, while non-soluble concentration generally increased, and soluble protein nitrogen generally decreased, especially the first two days of stress. These results indicate that moisture stress effectively reduced the net rate of incorporation of nitrogen into water soluble proteins. A major portion of the available reduced nitrogen (soluble non-protein) was apparently incorporated into non-soluble forms. Thus, non-soluble nitrogen content per plant increased while soluble non-protein nitrogen content remained constant. As stress became increasingly severe, relatively less nitrogen was incorporated into non-soluble nitrogen, resulting in a slight accumulation of soluble non-protein nitrogen per plant.

The explanation of changes in nitrogen fractions in Age I plants proposed above is further substantiated when the percentage contribution of each to total is examined (Figs. 11 and 13). Contribution of soluble protein nitrogen to total nitrogen decreased at almost the same rate as that of non-soluble nitrogen increase. Soluble protein nitrogen per plant was increasing; therefore, it appears that synthesis of non-soluble compounds increased at a much faster rate than that of water soluble proteins.

Changes in concentration of nitrogen fractions and relative contribution to total nitrogen were also compared in Age II plants. These data are reported in Fig. 14 and in appendix tables 8 to 13.

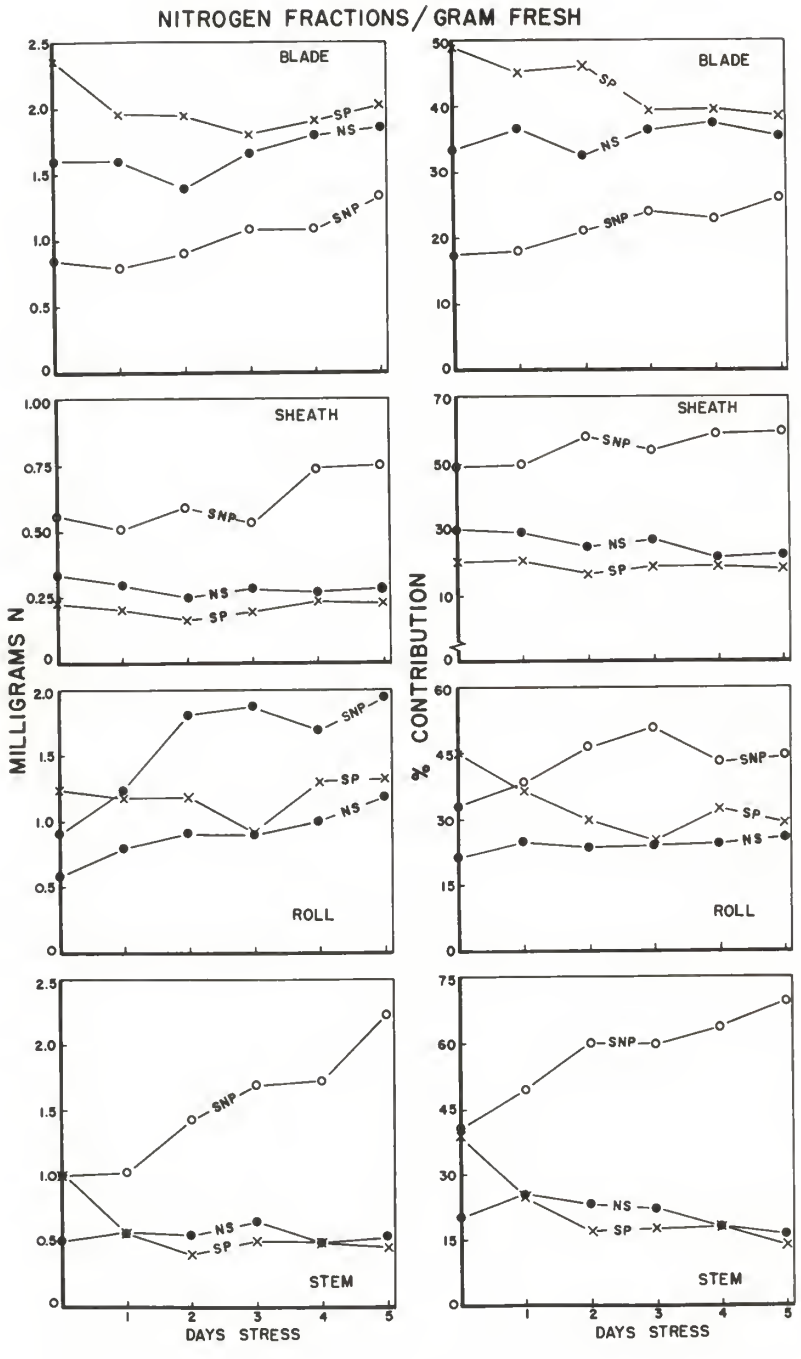


Fig. 14. Nitrogen fractions and their contribution to total nitrogen in different parts of Age II plants.

In contrast to the rapid increase in Age I plants, concentration of non-soluble nitrogen in Age II plants increased only slightly in the leaf blade and roll and remained constant or decreased slightly in the stem and sheath. Also, in view of the rapid decrease in water content of the blade and roll during stress (Fig. 9), much of the slight increase in concentration of non-soluble nitrogen in these plant parts likely reflects tissue dehydration rather than increased incorporation of nitrogen. Relative contribution of non-soluble nitrogen to total nitrogen was always constant or decreased only slightly. Thus, it appeared that stress in Age II plants reduced the rate of formation of non-soluble nitrogen.

Concentrations of soluble protein nitrogen in each part of Age II plants generally decreased during the first four days of moisture stress. For the remainder of the stress period, soluble protein nitrogen concentrations changed very little in the sheath and stem and increased slightly in the blade and roll. Percentage contribution of this fraction to total nitrogen decreased in each plant part. As with non-soluble nitrogen, these results indicate that an effect of moisture stress may have been reduced rate of incorporation of nitrogen into soluble protein.

A major difference in the influence of moisture stress between Age I and Age II plants was noted in changes in soluble non-protein nitrogen. In Age I plants, soluble non-protein nitrogen per gram fresh weight remained rather constant or increased only slightly until later stages of stress when a more rapid increase in concentration was noted. This pattern was repeated in the sheaths of Age II plants. However, in the roll, stem, and to a lesser extent in the blade of Age II plants, concentration of soluble non-protein nitrogen increased almost as soon as plants

were exposed to stress conditions. Percentage contribution of soluble non-protein nitrogen to total nitrogen increased at approximately the same rate as concentration in each plant part. In each plant part, changes in soluble non-protein nitrogen accounted for the greater part of the changes in total nitrogen.

Accumulation of soluble non-protein nitrogen concurrent with unchanged or reduced soluble protein nitrogen and non-soluble nitrogen in Age II plant parts suggests that metabolic systems may have been influenced differently by changes in moisture stress. It appeared possible that stress caused a decrease in rates of processes leading to formation of soluble protein and non-soluble forms of nitrogen. Thus, soluble non-protein nitrogen concentration would be expected to increase. The possibility also exists that increased soluble non-protein nitrogen and unchanged or decreased soluble protein nitrogen and non-soluble nitrogen resulted from hydrolytic processes rather than reduced synthesis. Proof of the existence of either process during moisture stress must be left to further experimentation.

Molybdenum

Molybdenum determinations were limited to Age I plants. Results are shown in Fig. 6 and in appendix table 1.

Molybdenum concentration varied only from 1.00 to 1.74 ppm during the entire stress period. Greatest response to moisture stress was noted from the second to the fourth days. Concentration increased during the first two days of exposure to stress, but decreased rapidly afterward to a level lower than the pre-exposure concentration.

Available data seem to indicate uptake of molybdenum continued when relative turgidity remained high and decreased in direct relationship to per cent turgidity. This decrease in concentration is difficult to explain but the possibility of increased dry weight and/or partial translocation cannot be overlooked.

This experiment measured the total amount of molybdenum in the plants; however, studies determining the amount of molybdenum associated with the nitrate reductase enzyme extract would seem more appropriate.

SUMMARY

Quantitative determinations were made of relative changes in nitrate reductase activity, levels of nitrate, various nitrogen fractions, total nitrogen, and molybdenum in corn plants under varying degrees of moisture stress. Two ages of plants were used (I) the 4-leaf stage when plants were 6 to 8 inches tall and (II) the 7-leaf stage when plants were approximately 28 inches tall. Determinations in Age I plants involved the entire plants. Age II plants were divided into the leaf blade, leaf sheath, leaf roll, and stem for analyses.

Exposure to high temperature, decreasing humidity, and decreasing moisture caused a sharp increase in moisture stress within all plants, as noted by both decreased relative turgidity and moisture content. Relative turgidity appeared to be a more sensitive measure of water stress.

Nitrate reductase activity decreased sharply in all plants with short exposure to stress conditions. The decreases were noted before changes in water status became evident. It was considered possible that nitrate reductase activity was influenced by changes in moisture stress too small to be measured by relative turgidity, or by factor(s) other than moisture stress.

Decreased nitrate reductase activity was reflected in accumulation of nitrate in all plants, particularly in the sheaths and stems of Age II plants, indicating that nitrate absorption and reduction may be separate and independent processes.

Total Kjeldahl nitrogen per plant increased to the level permitted by decreased reduction of nitrate and remained fairly constant thereafter.

Total nitrogen was separated into three parts: (1) non-soluble (water insoluble) nitrogen, (2) water soluble protein nitrogen, and (3) water soluble non-protein nitrogen. Effects of moisture stress on concentrations of the nitrogen fractions varied between ages of plants and among plant parts.

Soluble protein nitrogen concentrations were reduced immediately by moisture stress, regardless of age or part of plant analyzed. Relative contribution of soluble protein nitrogen to total nitrogen also decreased as stress continued although quantities per plant tended to remain constant. These results indicated that increased stress decreased and/or prevented further synthesis of water soluble proteins.

Another major effect of increased stress was noted in differences in changes in soluble non-protein nitrogen and non-soluble nitrogen as plants became older. As stress increased, non-soluble nitrogen concentrations increased rapidly in Age I plants, but increased at a slower rate or remained unchanged in Age II plants. Changes in concentration of non-soluble nitrogen in Age II plant parts were associated with degree of tissue hydration. These trends were also reflected in increased contribution of non-soluble nitrogen to total nitrogen in Age I plants and slight to no change in Age II plants.

In contrast, concentration of soluble non-protein nitrogen increased rather slowly in Age I plants until stress became very severe. Quantities per plant tended to increase throughout the stress period. In Age II plants, however, concentrations of soluble non-protein nitrogen generally increased earlier and more rapidly on exposure to stress. Contribution of soluble non-protein nitrogen to total nitrogen remained relatively unchanged in Age I plants, but rapidly increased in Age II plants.

Accumulation of soluble non-protein nitrogen in Age II parts, concurrent with unchanged or reduced soluble protein nitrogen and non-soluble nitrogen may have been caused by decreased synthesis, increased hydrolysis, or both.

General levels of molybdenum were extremely low. However, concentration in Age I plants decreased with increased moisture stress and appeared to be closely associated with decreased relative turgidity.

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APPENDIX

Table 1. Data obtained on fresh weight basis, in Age I tissue.

Days stress	Water %	Relative Turgidity (%)	NO_3-N	NO_2 (ug.)* (NR activity)	Total N mg.	Molybdenum ppm**
0	90.64	97.224	.5241	61.268	4.751	1,181
1	90.65	97.096	.6748	41.303	4.825	-
2	90.37	96.224	.5592	18.081	4.357	1.745
3	89.94	87.390	.5284	7.362	4.689	-
4	89.05	77.914	.5952	3.998	5.003	1.128
5	88.16	70.301	.6443	5.600	5.360	-
6	87.00	68.066	.7443	3.954	5.655	.996
7	85.93	64.533	.8476	3.922	6.380	-
LSD, .05	.52	4.568	.0376	2.104	.297	

* Nitrate reductase activity in micrograms of nitrite formed per 30 minutes incubation at 27°C.
** Calculated on dry weight basis.

Table 2. Various nitrogen fractions (per gram fresh weight) and their contribution to total nitrogen in Age I corn.

Days stress	NS-N* mg.	SP-N* mg.	SNP-N* mg.	Contribution to Total N (%)		
				NS-N*	SP-N*	SNP-N*
0	1.012	2.342	1.396	21.290	49.348	29.361
1	1.354	2.021	1.450	28.180	41.869	29.951
2	1.501	1.561	1.295	35.211	35.358	29.432
3	1.589	1.716	1.384	34.109	36.671	29.220
4	1.830	1.745	1.428	36.821	35.097	28.082
5	2.017	1.756	1.588	37.643	33.028	29.328
6	2.047	1.844	1.764	36.348	32.757	30.895
7	2.315	2.013	2.052	36.634	31.394	31.971
LSD, .05	.193	.206	.199	3.561	3.309	2.951

* NS-N = non-soluble (water insoluble) nitrogen; SP-N = water soluble protein nitrogen; SNP-N = water soluble non-protein nitrogen.

Table 3. Data obtained per plant basis in Age I tissue.

Days stress	NO_3-N mg.	NO_2 (ug.)* (NR activity)	NS-N** mg.	SP-N**	SNP-N** mg.	Contribution to Total N (%) NS-N**	SP-N**	SNP-N**	Total N mg.
0	.3187	36.325	.624	1.386	.845	21.654	48.655	29.691	2.856
1	.4363	26.555	.997	1.470	1.041	28.172	41.875	29.952	3.508
2	.5602	15.921	1.496	1.567	1.302	34.680	35.634	29.685	4.365
3	.6786	9.487	1.624	1.771	1.415	33.768	36.827	29.406	4.810
4	.7347	5.037	1.834	1.761	1.390	36.688	35.217	28.096	4.984
5	.7662	6.347	1.890	1.666	1.453	35.992	33.046	29.295	5.008
6	.8876	4.429	1.846	1.668	1.558	36.350	32.756	30.894	5.072
7	.8556	4.043	1.913	1.651	1.674	36.635	31.394	31.970	5.237
LSD, .05	.0386	1.526	.167	.215	.172	3.537	2.958	2.852	.339

* Nitrate reductase activity in micrograms of nitrite formed per 30 minutes incubation at 27°C.

** NS-N = non-soluble (water insoluble) nitrogen; SP-N = water soluble protein nitrogen; SNP-N = water soluble non-protein nitrogen.

Table 4. Percentage water in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	85.62	91.86	88.94	92.24	89.66
1	89.63	90.64	91.83	92.68	91.20
2	83.80	93.22	89.64	91.59	89.56
3	82.22	91.86	87.92	89.95	87.99
4	81.68	91.50	86.88	91.60	87.92
5	80.80	90.59	86.28	90.34	87.00
Ave.	83.96	91.61	88.58	91.40	

LSD, .05 Days = 2.45; Parts = 1.99; Days x parts = 4.89

Table 5. Total nitrogen in milligrams per gram fresh weight in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	4.812	1.097	2.728	2.556	2.798
1	4.358	1.014	3.210	2.158	2.685
2	4.213	1.004	3.900	2.376	2.873
3	4.532	1.059	3.684	2.856	3.033
4	4.766	1.245	4.001	2.702	3.178
5	5.198	1.265	4.444	3.226	3.533
Ave.	4.646	1.114	3.661	2.646	

LSD, .05 Days = .465; Parts .379; Days x parts = .929

Table 6. Nitrate in milligrams per gram fresh weight in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	1.004	1.928	.478	1.598	1.252
1	1.132	2.658	1.092	2.204	1.772
2	1.185	2.663	1.509	2.183	1.885
3	1.450	2.462	2.119	2.256	2.072
4	1.413	2.585	1.756	2.451	2.051
5	1.376	3.499	1.681	2.739	2.324
Ave.	1.260	2.632	1.439	2.238	

LSD, .05 Days = .296; Parts = .241; Days x parts = .593

Table 7. Nitrate reductase activity in micrograms of nitrite formed per 30 minutes at 27°C. per gram fresh weight in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	16.720	4.970	6.340	3.220	7.812
1	12.010	.677	1.404	.992	3.771
2	12.180	.466	.616	.806	3.517
3	12.270	.352	.481	.630	3.433
4	4.030	.272	.363	.432	1.274
5	1.625	.095	.266	.213	.550
Ave.	9.806	1.139	1.578	1.049	

LSD, .05 Days = 1.540; Parts = 1.258; Days x parts = 3.082

Table 8. Non-soluble (water insoluble) nitrogen in milligrams per gram fresh weight in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	1.614	.336	.586	.512	.762
1	1.604	.299	.795	.572	.818
2	1.384	.249	.905	.545	.771
3	1.656	.285	.895	,656	.873
4	1.790	.271	1.006	.491	.890
5	1.853	.285	1.179	.530	.962
Ave.	1.650	.288	.894	.551	

LSD, .05 Days = .149; Parts = .122; Days x parts = .298

Table 9. Per cent contribution of non-soluble (water insoluble) nitrogen to total nitrogen in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	33.50	30.36	21.56	20.37	26.45
1	36.62	29.28	24.82	25.58	29.08
2	32.80	24.84	23.49	22.90	26.01
3	36.48	26.96	23.93	22.40	27.44
4	37.45	21.68	24.68	18.19	25.50
5	35.48	22.57	26.33	16.36	25.18
Ave.	35.39	25.95	24.14	20.97	

LSD, .05 Days = 4.48; Parts = 3.66; Days x parts = 8.98

Table 10. Water soluble protein nitrogen in milligrams per gram fresh weight in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	2.358	.229	1.245	1.036	1.217
1	1.967	.205	1.179	.562	.978
2	1.943	.168	1.191	.399	.925
3	1.796	.192	.920	.508	.854
4	1.888	.236	1.298	.492	.978
5	2.015	.226	1.320	.460	1.005
Ave.	1.994	.209	1.192	.576	

LSD, .05 Days = .198; Parts = .162; Days x parts = .397

Table 11. Per cent contribution of water soluble protein nitrogen to total nitrogen in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	49.00	20.40	45.14	39.06	38.40
1	45.24	20.96	36.67	25.07	31.98
2	46.16	16.78	29.84	16.94	27.43
3	39.46	19.11	25.21	17.72	25.38
4	39.64	19.14	32.37	18.18	27.33
5	38.53	18.02	29.15	14.03	24.93
Ave.	43.00	19.07	33.06	21.83	

LSD, .05 Days = 4.48; Parts = 3.65; Days x parts = 8.95

Table 12. Water soluble non-protein in milligrams per gram fresh weight in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	.840	.556	.906	1.008	.828
1	.786	.510	1.237	1.024	.889
2	.886	.588	1.804	1.433	1.178
3	1.080	.530	1.869	1.693	1.293
4	1.088	.738	1.692	1.720	1.310
5	1.330	.754	1.945	2.235	1.566
Ave.	1.002	.613	1.576	1.519	

LSD, .05 Days = .106; Parts = .086; Days x parts = .211

Table 13. Per cent contribution of water soluble non-protein nitrogen to total nitrogen in different parts of Age II plants.

Days stress	Blade	Sheath	Roll	Stem	Ave.
0	17.50	49.30	33.30	40.57	35.17
1	18.14	49.77	38.50	49.34	38.94
2	21.04	58.38	46.67	60.16	45.56
3	24.06	53.92	50.85	59.88	47.18
4	22.91	59.18	42.96	63.64	47.17
5	25.98	59.40	44.52	69.61	49.88
Ave.	21.60	54.99	42.80	57.20	

LSD, .05 Days = 5.68; Parts = 4.64; Days x parts = 11.36

TRENDS IN NITRATE REDUCTION AND NITROGEN FRACTIONS IN CORN PLANTS DURING MOISTURE STRESS

by

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Changes in the levels of nitrate, various nitrogen fractions, total nitrogen, molybdenum, and relative nitrate reductase activity were determined in two ages of corn under exposure to increased moisture stress. The ages analyzed were: (I) the 4-leaf stage when plants were 6 to 8 inches tall and (II) the 7-leaf stage when plants were approximately 28 inches tall. Determinations on Age I plants included the entire plant while Age II plants were divided into the leaf blade, leaf roll, leaf sheath, and stem.

Moisture stress within plants was noted by decreased relative turgidity and reduced moisture content. Moisture stress increased with exposure to high temperature, decreased humidity, and decreased moisture.

Nitrate reductase activity was sharply decreased before extensive exposure to stress had occurred and in most cases concentrations were extremely low during later stages of stress. However, activity in the leaf blade was always higher during the sampling period than in other plant parts.

Low nitrate reductase activity was associated with nitrate accumulation in both ages during increased moisture stress. In Age II plants, concentrations were highest in the sheath and stem. These trends indicate nitrate absorption and reduction may be separate and independent processes.

Total nitrogen concentrations increased with exposure to moisture stress in both ages. Increase in total nitrogen concentration at later stages of stress was attributed to decreased hydration of tissue, since on a plant basis total nitrogen remained constant during these stages.

Total nitrogen was further divided into non-soluble nitrogen, soluble protein nitrogen, and soluble non-protein nitrogen, based on solubility in

water. Effects of moisture stress on these fractions varied greatly between ages and parts analyzed.

Concentrations of soluble protein nitrogen decreased greatest during early periods of moisture stress in both ages, although quantities per plant remained relatively constant. Per cent contribution of soluble protein nitrogen to total nitrogen also decreased most during initial moisture stress periods and indicated that synthesis of soluble protein nitrogen was decreased or prevented by moisture stress.

Non-soluble nitrogen concentrations increased more rapidly in the younger plants than in the older plants, and any increases appeared closely associated with degree of tissue hydration. These trends were reflected in increased contribution of non-soluble nitrogen to total nitrogen in Age I plants, and slight to no change in Age II plant parts.

Increases in concentration of soluble non-protein nitrogen occurred in Age I plants only when stress became severe in contrast to an immediate and more rapid increase in Age II plant parts. Quantities per plant tended to increase throughout the stress period. Contribution of soluble non-protein to total nitrogen remained relatively unchanged in Age I plants, but rapidly increased in Age II plants. This increase in concentration of soluble non-protein nitrogen along with relatively no change in soluble protein nitrogen and non-soluble nitrogen may be explained by decreased synthesis and/or increased hydrolysis of protein.

Levels of molybdenum were low in Age I plants and concentrations decreased with moisture stress.