NITRATE ACCUMULATION IN PEARL MILLET AND SUDANGRASS

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Preface

This study explores the relationship between nitrate concentration in Piper sudangrass, a sorghum x sudangrass hybrid and three pearl millets and nitrogen fertilizer rates of 0, 90, and 180 kg ha^{-1}, clipping height, time of day of harvest, and seasonal growth stage. The objectives were to evaluate plant nitrate concentrations as affected by these parameters.

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CHAPTER I

INTRODUCTION

Sudangrass (Sorghum bicolor (L.) Moench) and pearl millet (Pennisetum americanum (L.) Leeke) are grown extensively for summer forage in the southern Great Plains and the southeastern United States, respectively. Both provide an excellent source of forage when grown under ideal climatic and cultural conditions. Adverse environmental conditions can cause excessive nitrate concentrations in these forages. The degree to which nitrate accumulates in plants is affected by factors such as low light, extremes in temperature, drought, species, developmental stage, and time and rate of N application (Wright and Davison, 1964; Terman, Noggle and Hunt, 1976). Excessive nitrate in forages is known to induce death to ruminant animals, while lower, non-lethal levels reduce rate of gain or milk production and can cause abortion. Potentially toxic levels of nitrate in forage have been described by Reid and Jung, 1973 as approximately 15 g kg^{-1}, while Tucker et al., 1961 and Gillingham et al., 1969 have suggested that from 8.8 to 13.2 g kg^{-1} is dangerous depending on animal size. Nitrate poisoning of ruminants is often a serious problem in Texas and Oklahoma, particularly when the summer growing season is hot and dry (Edwards and McCoy, 1980).

Nitrate accumulation in forages has been studied in corn (Zea mays L.), (Hanaway and Englehorn, 1958; Gonske and Keeney, 1969), bermudagrass [Cynodon dactylon (L.) Pers.], (Lovelace, Holt and Anderson, 1968),

western wheatgrass (Agropyron smithii Rydb.), green needlegrass (Stipa viridula Trin.), (White and Halvorsen, 1980), and small grains (Moeller and Thurman, 1966). Although pearl millets generally contain low levels of prussic acid (Krejsa, et al., 1984), they may extract more nutrients from soil (Smith and Clark, 1968) and accumulate greater amounts of nitrate than the sudangrasses and the sorghum x sudangrass hybrids (Clark, Hemken and Vadersall, 1965; Schneider and Clark, 1970).

Many forages have been shown to contain greater concentrations of nitrate in stems than in leaves (Baker and Tucker, 1971; Crawford, Kennedy and Johnson, 1961). Nitrate transport from the roots to apical portions of the plant occurs via the xylem. Nitrate is commonly reduced to nitrite in the cytoplasm of the mesophyll cells within the leaves (Mellor and Tregunna, 1971). Thus, levels of nitrate can be expected to be higher in the stem than in the leaves.

No data seem to be available on nitrate concentration as affected by harvest methods for pearl millet and sudangrass grown in the drought prone southern plains. Accordingly, the objectives of these studies were to evaluate nitrate concentration in five sudangrass and pearl millet forages as affected by N fertilization and to evaluate clipping height, date of clipping, and time of day as a means to reduce nitrate concentration in the forage.

CHAPTER II

LITERATURE REVIEW

A. Toxicity of Nitrate to Animals

1. Ruminant vs. Non-Ruminant Susceptibility

Ruminant animals are more often associated with nitrate (NO_3^-) poisoning than the non-ruminants for two reasons. First, the nature of the ruminants diet compared to that of the non-ruminants is such that ruminant animals are more commonly fed high fiber forage diets, which may have a propensity for accumulation of high levels of nitrate. Non-ruminants are generally unable to derive as much nutritive advantage from high fiber forage diets as ruminants and are, therefore, fed feeds which are generally low in fiber and generally not associated with nitrate accumulation. There is a pronounced physiological difference in digestive tract anatomy and physiological metabolism which is basic to the difference in both feed usage and nitrate absorbtion by the two groups. Second, these physiological differences affect the mechanism for nitrate absorption by the animal. The toxicity problem to the animal is caused by the nitrite ion.

A comparison of the ruminants' digestive tract to that of the non-ruminants' tract is paramount in understanding why ruminants are more susceptible to nitrate poisoning than non-ruminants. The ruminants' digestive tract consists of the mouth, esophagus, rumen, reticulum,

omassum, abomassum, small intestine, cecum, large intestine, and rectum. Ruminants depend on a vast microbial population to break down high fiber feeds into constituents the animal can absorb and utilize. The primary site of major microbial population and action, the rumen, is anterior to the abomassum, or "true stomach." Some microbes do exist in the small and large intestines; however, most microbes are unable to tolerate the low pH environment of the abomassum, hence they are generally confined to the more basic environment of the rumen.

Non-ruminants, for all practical purposes, do not utilize microbial populations for digestion of feeds, hence their inability to effectively utilize high fiber forages. Their primary digestion occurs through mastication and denaturation via acid, constituting an environment non-conducive to microbial life in the stomach.

Digesta passage rate through the ruminants' digestive tract is predicated on its location. Passage through organs posterior to the true stomach is much faster than passage through those organs anterior to the abomassum. Passage of digesta through a non-ruminant's digestive tract is faster than passage through a ruminant's digestive tract. Passive absorbtion of ions occurs primarily in those organs where rate of passage is slow, while active absorbtion occurs primarily in the small intestines. Nitrate (inherently harmless) is passively absorbed in the digestive tracts of both non-ruminant and ruminant animals. Reduction of nitrate to nitrite within the gastrointestinal tract is attributed solely to microorganisms. Nitrate consumed by monogastric animals undergoes little reduction to nitrite until it reaches the large intestine. But, rate of passage is generally not slow enough to instigate toxic reactions within the animal. Ruminants are especially

susceptible to nitrate toxicity because much metabolism and absorbtion of nitrite and its reduction products occur in the rumen, anterior to the stomach (Bradley, Eppson, and Beath, 1939). Rate of passage, microorganism location and population, and type of feed lead to distinct contrasts in NO_3^- reduction and assimilation between the non-ruminants and ruminants.

2. Metabolism of NO_3^- by Animals

As a consequence of the nitrate reducing capacity of microorganisms in the rumen, and other mentioned factors, ruminants are especially vulnerable to nitrate poisoning. Kemp et al. (1977, 1978) and Geurink et al. (1979) have reported four major factors which directly influence the formation of nitrite in the rumen fluid and methemoglobin in the blood: the amount of nitrate consumed, the rate of nitrate intake, the rate of release of nitrate from the roughage into the rumen fluid, and, adaption to a higher nitrate level by the rumen microbes. The reducing capacity of the microbes within the animal probably increases during the first days after nitrate dosing to reach a maximum after five days (Van Dijk et al., 1983) Nitrate is reduced to ammonia and nitrite is an intermediate in this reaction (Horner, 1982). As a consequence, both nitrate and nitrite may be absorbed into the bloodstream. The nitrite ion is more toxic (10x) to the animal (Burrows, 1982). As the nitrite ion contacts erythrocytes, it oxidizes hemoglobin iron from an active ferrous form to the ferric state yielding methemoglobin. Methemoglobin is not capable of oxygen carrying duties. In effect, the nitrite ion blocks or fills the space that oxygen would otherwise occupy. This occurrence reduces the oxygen carrying capacity of the blood, often

times resulting in asphyxiation of the animal.

3. Types of Toxicity

Nitrate toxicity may be of two types: acute or chronic. Acute toxicity usually refers to the sudden, lethal expression of nitrate intoxication. Chronic toxicity is more nebulous in its physical expressions, and symptoms may become evident or appear over a longer period of time.

Acute Toxicity. Initial indications of acute toxicity due to nitrate poisoning are the appearance of grayish to brownish lesions on the white areas of the skin and the non-pigmented mucous membrance of the mouth, nose, eyes, and vulva. As the poisoning progresses, animals may exhibit labored respiration, muscle tremors, excessive salivation, frequent urination, staggering gait, rapid pulse, and ataxia (Horner, 1982). The rapid onset of these symptoms may be followed by coma and death of the affected animal. Post mortem examination of mucous membranes and blood will reveal a characteristic chocolate brown discoloration.

Chronic Toxicity. Sublethal levels of nitrate ingestion can result in reduced rate of gain or milk production, vitamin A and iodine deficiency (Thyroid dysfunction), and absortion (Beeson, 1964; Case, 1957). The well known vasodilating properties of the nitrites appear to play a very limiting role in nitrate-nitrite toxicosis (Burrows, 1982). However, other researchers have shown a clear decrease in blood pressure since the nitrate ion has a direct relaxing effect on the smooth muscle fiber of smaller blood vessels (Ashbury and Rhode, 1964; Assali and Brinkman, 1973).

B. Localization of Nitrate Within the Plant

Intracellular nitrate compartmentalization is now known to occur. The existence of two separate nitrate pools, namely the metabolic pool serving as a substrate for nitrate reductase, and a storage pool situated in the vacuole and unavailable for nitrate reduction, is an accepted concept for the intracellular distribution of nitrate in higher plants (Jones and Sheard, 1975; Hog and Hartvigsen, 1983; Ferrari et al., 1973; Pate, 1973). Factors affecting permeability of cell membranes, namely light, carboydrates and plant hormones, can affect the substrate supply to the nitrate reducing system (Hog et al., 1983).

Accumulation of nitrate within the mesophyll cells of plants has been found to occur at up to thirty times that found in bundle sheath cells (Mellor and Tregunna, 1971). The stem of the plant has also been implicated as a storage area for reduced nitrogen (Crawford et al., 1961; Krejsa et al., 1983; Steer, 1982; Terman et al., 1974; Crawford et al., 1960). This may be due to the fact that most NO_3^- not reduced in the roots is translocated through the xylem in the stem to the area of primary reduction, leaf mesophyll cells. Nitrate accumulation in the stem has been found to increase, while root accumulation decreased, as NO_3^- was supplied in greater quantities to the growth medium (Steer, 1982). Pate (1973) has found that surplus nitrogen arriving from the root may be stored in the shoot, from where it is drawn upon extensively if uptake by the root fails to keep pace with the shoots demands for nitrogen.

Thus a general picture evolves whereby: 1) NO_3^- is concentrated in the stem because of both its transport through xylem vascular tissue and storage capabilities of cell vacuoles within the stem; 2) many plants accumulate high levels of NO_3^- in the roots as this is the site

of NO_3^- ion uptake into the plant; 3) nitrate is concentrated in mesophyll cells more than in bundle sheath cells; and 4) NO_3^- is compartmentalized intracellularly into two distinct pools, a metabolically active pool and a storage pool of limited availability which is located in the vacuole of the cell.

C. Metabolic Pathway of Nitrate Within the Plant

1. Pathway of Assimilation and Metabolism

The assimilatory reduction of nitrate by plants is a fundamental, biological process in which an oxidized form of nitrogen is reduced to ammonia. Ammonia is then combined with carbon skeletons to form the different nitrogenous compounds used by the plant. Most higher plants growing in soil obtain their nitrogen in the form of nitrate. It has been observed, however, that uptake of NO_3^- from solutions containing both NO_3^- and ammonia begins only after ammonia has been exhausted or its concentration greatly reduced (Bayley et al., 1972).

Epidermal and cortical cells of the root are the main sites of NO_3^- ion uptake (Laties, 1969). A proportion of these NO_3^- ions is subsequently diverted into vaculoes of the cells in these regions, a portion is used by the root for its growth, while the remainder diffuses along concentration gradients in the root cytoplasm, through the endodermis to cells in the stele. From the stele, the NO_3^- ions are passed to the xylem where they are then free to be translocated to the other areas of the plant.

Other nitrogen containing compounds are produced by reduction of nitrate. Commonly produced compounds are the amides glutamine and asparagine and various other closely related amino acids. A few

species reduce nitrate to alkaloids, ureides, or certain other unusual non-protein amino acids (Pate, 1973). In plants where a high percentage of nitrate, relative to other nitrogenous compounds, is transported through the xylem from the roots, a general summary is useful in describing the pathway.

Nitrate is transported through the xylem where it is then channeled into either a storage nitrate pool, located in the vacuole of the cell, or a metabolically active nitrate pool located in the cell cytoplasm. Nitrate from the metabolically active pool is reduced to nitrite through the action of the enzyme nitrate reductase. Nitrite is further reduced to NH_3 via the enzyme nitrite reductase and from NH_3 to glutamine, glutamate, and various amino acids. The final products glutamine, glutamate and amino acids can be used immediately for protein synthesis or can be exported to the phloem for translocation to other areas of the plant.

D. Nitrate Reductase Activity

Nitrate reductase is frequently referred to as a cytoplasmic enzyme (Dalling, 1972). The nitrate reductase of higher plants catalyzes the reduction of nitrate to nitrite by reduced pyridine nucleotides following the equation given below.

$$NO_3^- + NAD(P)H + H \text{ -----> } NO_2^- + NAD(P) + H_2O$$

In the pathway NO_3^- --> NO_2^- --> amino acids, reduction of nitrate to nitrite by the enzyme nitrate reductase (NR, EC 1 . 6 . 6 . 1) is believed to be the rate limiting step (Beevers and Hageman, 1969). EC 1 . 6 . 6 . 1 is specific for NADH and corresponds to the type of enzyme present in the leaves of most higher plants (Kuo et al., 1980). According to

the specificity for the electron donor, NADH or NAD(P)H, two main types of assimilatory nitrate reductase are known: (a) ferredoxin-dependent nitrate reductase, present in blue-green algae and photosynthetic bacteria, and (b) pyridine nucleotide-dependent nitrate reductase, which is found in eukaryotic organisms. The specificity difference between the two systems is due to differences in their prosthetic groups and physiochemical properties (Gerrrero et al., 1981). The pyridine nucleotide-dependent enzyme of higher plants is a soluble enzyme, while the ferredoxin-nitrate reductase of prokaryotes appears to be tightly bound to photosynthetic membranes (Losada et al., 1979).

Although most higher plant species appear to be specific for NADH as opposed to NAD(P)H as donor (Beever et al., 1969), some degree of flexibility exists in plants. The simultaneous presence of two different nitrate reducing enzymes, NADH and NAD(P)H dependent, has been demonstrated for soybean leaves (Campbell, 1976). The presence of two nitrate reductases showing specificity for both pyridine nucleotide electron donors has also been discovered in rice seedlings (Shen et al., 1976).

There are three prosthetic groups (also referred to as coenzymes or metal ions) associated with the NAD(P)H specific nitrate reductase; cytochrome b-557, FAD and molybdenum. The prosthetic group composition of NADH dependent nitrate reductase consists of FAD, a heme group (cytochrome b type), Mo, and two HCN binding sites. Cyanide binding sites are thought to play possible regulatory roles in nitrate reductase activity (Solomonson and Spehar, 1977). The pathway of electron transfer from NAD(P)H or NADH to nitrate reductase in eukaryotes is:

NAD(P)H --> [FAD --> cytochrome b-557--> Mo] --> NO_3^-.

NADH for nitrate reductase is derived from the metabolism of 3-phosphoglyceraldehyde (3-PGA) produced during photosynthesis. However, NADH which has been generated in the citric acid cycle (respiration) has been suggested to be utilized for in vivo nitrate reduction in sorghum seedling leaves. Additionally, NADH generated in the cytoplasm during glycolysis can be oxidized in mitochondria and conversely, mitochondrial NADH can be used for nitrate reductase in the cytoplasm (kadam et al., 1980).

1. Substrate Induction

There is general agreement that NO_3^- enhances nitrate reductase activity. In higher plants, nitrate reductase is usually regarded as a substrate inducible enzyme (Hewitt, 1975; Srivastava, 1980). Nevertheless, in higher plant systems, considerable enzyme levels are sometimes found in the absence of nitrate, as is the case for maize seedlings (Mengel et al. 1983). As soon as the enzyme begins to reduce NO_3^-, conditions are produced (pH increase or accumulation of organic anions) which promote its further activation or synthesis (Mengel et al., 1983; Heimer et al., 1979).

E. Factors Affecting Nitrate Reductase Activity

Nitrate reductase levels have been shown to fluctuate in response to changes in environmental conditions such as light, temperature, pH CO_2, and oxygen tensions, water potential, nitrogen source, and other factors, changes that usually also influence the capacity of the organism to assimilate nitrate (Beevers and Hageman, 1969; Hewitt, 1975).

Accumulated nitrate represents the balance between uptake and reduction by nitrate reductase.

1. Water Stress

A decrease in water potential below -0.4 to -0.2 mPa causes a decrease in nitrate reductase activity in maize and many other plants (Morilla et al., 1973; Ackerson et al., 1971). Sung (1981) has reported that nitrate reductase activity fell sharply in sweet potato, a plant relatively insensitive to water stress, when leaf water potential dropped to between -0.7 and -1.4 mPa. Inhibition of enzyme synthesis and an inhibition of enzyme induction was implicated as the reason for reduced nitrate reductase activity due to a decrease in leaf water potential. A drop in nitrate reductase activity was clearly caused by a decrease in the rate of enzyme synthesis at low leaf water potentials in a study conducted by Morilla et al. 1973.

Protein synthesis within the plant is also adversely affected by water stress. Morilla et al. (1973) found that a decline in polyribosomal content preceeded the reduction in nitrate reductase activity consistent with a reduction in the rate of synthesis of the enzyme. Additionally, the recovery of a large percentage of polyribosomes and nitrate reductase activity after one hour occurred concurrently (Morilla et al., 1973). Mehta and Srivastava (1980) reported that an increase in nitrate reductase activity by NO_3^- ions is dependent upon protein synthesis, a function of ribosomes.

2. Temperature

Variation in temperature during seedling growth affects enzyme

levels in plants. Inactivation of nitrate reductase activity at temperatures lower than optimum is not as drastic as at higher temperature. However, cooling of roots has been shown to decrease the flux of NO_3^- from the roots to the leaves, thereby indirectly affecting nitrate reductase activity. Nitrate reductase activity was found to decline in leaf tissue after a period of time following cooling of the roots (Shaner and Boyer, 1976). Indeed, Shaner and Boyer found that nitrate flux to the leaves from the roots plays a much larger regulatory role than the leaf NO_3^- content in controlling the level of nitrate reductase activity in intact plants.

High temperatures have a more pronounced effect on nitrate reductase activity. The cardinal temperature range for optimum nitrate reductase activity in sorghum appears to be from between 35-40C. Temperatures above 40C result in a rapid decrease in nitrate reductase activity. Differences in reduction of nitrate reductase activity due to high temperatures have been shown to vary depending on cultivar (Choppa, 1983). Excessive temperatures can cause a denaturation of protein and perhaps the production of an enzyme inhibitor.

3. Carbon Dioxide

Since CO_2 fixation by the Calvin cycle is the ultimate source of carbon skeletons for the fixation of ammonia produced by assimilatory nitrate reduction, little or no fixation of ammonia could occur in the absence of CO_2. It is reasonable that these two processes be coordinately regulated. When the rate of CO_2 fixation is low, the accumulation of potentially toxic intermediates of nitrate assimilation such as nitrite, hydroxylamine, and ammonia could be produced. When

the rate of CO_2 fixation is high, the formation of sufficient ammonia for the synthesis of organic compounds would be ensured (Solomonson and Spehar, 1977).

4. Light

The nature of the stimulation by light of nitrate assimilation in photosynthetic cells has been a matter of controversy for a long time. Light has been said to be essential for the substrate induction of nitrate reductase (Travis et al., 1970), yet in a later study, Travis et al. (1971) demonstrated the induction of nitrate reductase in young maize seedlings in the dark. Lillo (1983) has observed that plants transferred to light had a dramatic increase in nitrate reductase activity during the first hour, then with nitrate reductase activity remaining fairly constant. Upon removal from light, nitrate reductase activity decreased during the first hour of darkness then remained constant. Aslam et al. (1981) suggested that light was required for the transfer of NO_3^- from the storage pool to the metabolically active pool. Assuming that nitrate reduction occurs in the cytoplasm outside the chloroplast, and in order to explain how the photosynthetically generated reducing power comes out of the chloroplast and is transferred to the electron donor for nitrate reduction, the participation of a variety of shuttle systems has been proposed (Krause et al., 1976). Others have concluded that photosynthesis is involved in induction of nitrate reductase activity either directly (Chen et al., 1969), or by increasing cytoplasmic protein synthesis (Travis et al., 1970). However, Jolly and Tolbert (1978) have concluded that nitrate reductase levels in light and dark are regulated by the relative activities of

specific inhibitors and activators. They have proposed that the inhibitor is formed in the dark and is reversibly inactivated by light. Aparicio et al. (1976) have demonstrated the photoreactivation of nitrate reductase with light of different colors. Reactivation was most pronounced following illumination with either blue or white light, while red light had no reactivating effect. This inhibitor may be a compound analagous to the protein phytochrome which absorbs red and far red light and affects certain plant functions e.g., seed germination and inhibition of flowering (Knypl, 1979). ATP synthesis in photosynthetic tissue can have a positive effect on general protein synthesis, and hence the production of nitrate reductase. At this point it is not known whether the regulation of nitrate reductase is due to enzyme synthesis or activation-inactivation.

5. Nutrients

The efficacy of various inorganic salts and ions on nitrate reductase activity has been thoroughly studied. Mengel et al. (1983) have pointed out that an addition of bicarbonate to the nutrient solution of maize seedlings resulted in a significant increase of the nitrate reductase activity in the roots. Bicarbonate, like OH^-, increase pH and promotes the synthesis of organic anions, and so, provides circumstantial evidence that alkaline conditions and/or organic anions have a direct impact on nitrate reductase activity.

It is widely accepted that an increase to the plant in available nitrogen will result in a concomittant increase in nitrate reductase activity, and also influence the capacity of the plant to assimilate NO_3^- (Srivastava, 1980; Beevers, 1969). However, the type of nitrogen

supplied is significant in that ammonia has been found to inhibit nitrate reductase activity either by limiting the uptake of nitrate (Bayley et al., 1972) or by interfering with its synthesis (Orebamjo et al., 1975).

Baker and Tucker (1971) reported that nitrate concentration in wheat leaves was reduced with the application of 15 kg ha^{-1} of phosphorous. However, phosphorous rates beyond that necessary for maximum grain production did not further reduce the nitrate content of the forage. In this case, phosphorous did not appear to influence nitrate reductase activity; rather, it allowed for the normal production of grain by the forage. Grain contains many nitrogenous compounds which are thought to be produced as a result of nitrate reduction.

Potassium is known to be involved in stomatal regulation and cellular adaption to osmotic stress, that is, osmoregulation, two factors which are closely coupled with water stress resistance (LeRudulier et al., 1984). Water stress can adversely affect protein synthesis and nitrate reductase activity as well as other physiological processes of the plant.

Nitrate reductase activity has been reported to rise in plants 24 hours following an application of potassium, and to help maintain nitrate reductase activity in the plant for 48 hours following application (Khanna-Chopra et al., 1980). Others have suggested that potassium stimulates nitrate translocation out of roots, resulting in a loss of root nitrate reduction. The decrease in root reduction of nitrate was thought to be a consequence not only of more nitrate translocation from the root to the xylem, but also a related decrease in availability of substrate (NO_3^-) at the sites of reduction (Blevins

et al., 1978). Indeed, this has been demonstrated by other researchers. Rufty et al. (1981) have shown that the activity of nitrate reductase is not the sole determinant for the quantity of nitrate which becomes reduced. Decreased nitrate reduction in roots in the presence of potassium could reflect a change in the location of nitrate uptake along the root relative to the position of maximal nitrate reductase activity; or, in the presence of potassium, a larger proportion of the tissue nitrate is sequestered in storage compartments (vacuoles) away from nitrate reduction sites (metabolically active pools).

Molybdenum, constituting a prosthetic group of the nitrate reductase enzyme, has significant effects on nitrate reductase activity. Nitrate reductase activity in molybdenum deficient plants is low but increases rapidly when molybdenum is supplied exogenously (Merkel et al., 1975). Nitrate reductase activity has been correlated positively with molybdenum in citrus leaves (Shake and Boyer, 1976).

The nitrate reductase enzyme contains an SH group; therefore, a deficiency of sulfur causes a decrease in nitrate reductase activity. Sulfur deficiencies have been reported to cause a decrease in nitrate reductase activity in maize (Friedrich et al., 1978). However, when sulfur is not limiting, its supply has no effect on nitrate reductase activity.

6. Herbicides

There are 134 herbicidal compounds listed in the 1968 "British Weed Control Handbook," 40 of which kill weeds by inhibiting photosynthesis (Buchel, 1972). The urea, 2-triazine and uracil herbicide

groups contain constituent compounds which are commonly known to inhibit the Hill reaction (splitting of water) in photosynthesis. The primary site of inhibition for many of the s-triazine herbicides is on the reducing side of photosystem II; the electron transfer step between the primary electron acceptor (Q) and the plastoquinone pool of the electron transport chain (Brewer et al., 1979; Machado et al., 1978; Yang and Bingham, 1984). In addition, the triazine herbicides have been shown to inhibit ATP synthesis which is a product of photosystem II (Thompson et al., 1974;) Trebst and Wietoska, 1975). ATP is used as an energy source for protein synthesis, a process closely related to the production of nitrate reductase, within the plant.

Some researchers have reported that an increase in nitrate reductase activity is an effect induced by the triazine compounds (Klepper, 1979; Fedtke, 1972). Fedtke (1972) has correlated decreases in photosynthesis with increases in nitrate reductase activity, and increased nitrate concentration in plants. Nitrate reductase is known to be substrate inducible, that is induced by nitrate, hence the increased nitrate content of the treated plants could be the cause for increased nitrate reductase activity. Other researchers have demonstrated an increased uptake and accumulation of nitrate in plants treated with s-triazines (Fedtke, 1972; Fink and Fletchall, 1967; Gramlich and Davis, 1967).

Metribuzin, an asymetrical triazine has been shown to induce accumulation of large amounts of nitrate in plant tissue (Fedtke, 1972). Certain hard red winter wheat cultivars have shown different responses to metribuzon applications. In one study, 'Lindon' and 'Vona' were more sensitive than were 'TAM W 101' or 'Osage' which

were the most tolerant of 15 tested hard red winter wheat cultivars (Runyan, McNeil, and Peeper, 1982).

The mechanism of resistance or tolerance, reported with annual grasses belonging to the genera Digitaria, Panicum, Setaria, and Sorghum are based on the rapid detoxification of the triazines to non-toxic metabolites (Jensen et al., 1977). The mechanism of resistance or tolerance of certain plant species is based on the differential function of chloroplasts as related to the inhibition of the Hill reaction (Machado et al., 1977).

7. Nitrate Reductase Inactivating Enzymes

There is wide evidence to support the claim of the presence of nitrate reductase inactivating enzymes in the roots of both maize (Zea mays) and rice (Oryza sativa). An enzyme responsible for the specific inactivation of NADH-nitrate reductase in the cytoplasm of maize and rice roots has been reported (Wallace, 1973; Yamaya et al., 1980). Yamaya et al. (1980) have concluded that the protein-like macromolecule in corn inactivates the NADH-cytochrome portion of the NADH-nitrate reductase complex. This macromolecule from corn was also found to inactivate nitrate reductase activity in the leaf by Wallace (1973). Later work by Wallace has shown that the inactivating level increases with seedling age in corn leaf tissue (Wallace, 1975). These enzymes appear to cause nitrate reductase inactivation through binding to the enzyme protein (Yamaya et al., 1980), and this action may be involved in reversible activity changes in response to light-dark transitions (Jolly and Tolbert, 1978; Sherrard et al., 1979). Casein, a milk protein, was found to inhibit the action of the

inactivating enzyme, in effect stabilizing nitrate reductase activity by preventing the action of the nitrate reductase inactivating enzyme (Wallace, 1975).

C. Cultural Practices Affecting Nitrate Accumulation

In a cause-and-effect relationship, nitrate accumulation appears to be an effect of a given causal factor(s). In many cases, it appears that less than ideal growing conditions adversely affect the labile nitrate reductase enzyme thereby curtailing or reducing nitrate reductase activity. This allows nitrate to accumulate in the plant without being reduced to nitrite. Under optimum growing conditions, there is usually no excessive accumulation of nitrate by either pearl millets or sudangrasses, unless excessive amounts of nitrogen fertilizer are applied.

1. Nitrogen Fertilization

It is widely accepted that an increase in the amount of applied nitrogen fertilizer will result in an increase in nitrate accumulation by many plants (Crawford et al., 1961; Terman et al., 1976; Murphy and Smith, 1967; Lemon and McMurphy, 1984; Gonske and Keeney, 1969; Moeller and Thurman, 1966; Hojjati et al., 1973; George et al., 1972; Fribourg and Loveland, 1978; White and Halvorson, 1980; Clark et al., 1966; Sumner et al., 1965). It is possible that under such fertility conditions the plant assimilates more nitrate than it can reduce, storing excess nitrate in storage pools. Thus, the plant may never reduce all its stored nitrate, unless uptake of nitrate from soil declines or ceases. A plant which has existing large stores of nitrate and which encounters physiologically adverse growing conditions may

not be able to convert the excess stored nitrate to nitrite.

Nitrogen in most cases has been shown to increase yields and is therefore widely used. A continual problem occurs in attempting to supply adequate amounts of nitrogen fertilizer for realization of potential yields without supplying so much that the plant needlessly stores excessive nitrate. Even the most carefully adjusted fertilization regimen can be complicated by inclement climatic conditions which adversely affect nitrate reductase activity. Split applications of small amounts of nitrogen fertilizer have been found to maximize yield while minimizing nitrate content in both forage and hay (Ealig and Hagemann, 1981; McCreery et al., 1966).

2. Stage of Maturity

Plants harvested at an advanced stage of maturity have been found to contain less nitrate than those plants harvested at a less mature age (Terman et al., 1976; Murphy and Smith, 1967). Nitrate reductase activity has been shown to be low in the glumes and upper leaf blades of plants during ear development. This has been suggested to result from translocation barriers to nitrate movement to the ears, or a preferential flow of nitrate to the leaf blades (Chatterjee et al., 1980). Gul and Kulp (1960) found that nitrate levels increased to a high at 25% flowering and then decreased to a low during 50% hard dough stage. Various factors are believed responsible for the decline in nitrate levels as the plant matures. First, nitrate is being converted to various other nitrogenous compounds which the plant is utilizing for seed production. Uptake of nitrogen by the roots of plants has been shown to be curtailed during flowering, and that which is taken up is

assimilated by various other plant parts (Pate, 1973; Chatterjee et al., 1980).

3. Height and Frequency of Harvesting

Researchers have found that nitrate levels in harvested forage were less when the cutting height was raised. Burger and Hittle (1967) found that nitrate in all harvested varieties was higher when plants were cut at 7.6 cm than when cut at 15.2 cm. McCreery et al. (1966) found that nitrate was highest in harvested forage when seven eighths of the plant was harvested vs. when one third of the plant was harvested. This is possible since a significant amount of stored nitrate and nitrate in-transit via xylem vascular tissue is left in the stubble, in the field, and is not part of the harvested forage. McCreery et al. (1966) have also demonstrated that forage harvested at two or three week frequencies was higher in nitrate than forage harvested every four weeks, with forage harvested at five week frequencies being lowest in nitrate. This is likely due to the advancing stage of plant maturity encountered at the four and five week frequency cuttings.

CHAPTER III

MATERIALS AND METHODS

In 1982 and 1983 three field experiments were conducted at Agronomy Research Stations located at Perkins and Lahoma, Oklahoma; once each year at Perkins (P82, P83) on different locations, and once at Lahoma (L82) in 1982. The design of all experiments was a randomized complete block with a 5 x 3 factorial arrangement of five forages and three nitrogen (N) fertilizer rates (0, 90, and 180 kg ha^{-1}) with four replications. All of the N fertilizer was supplied as ammonium nitrate and was surface applied (broadcast) on the seeding date. The five forages were three cultivars of pearl millet, (Gahi-3, Mil-hy, and Tifleaf-1), a 'Redland' x 'Greenleaf' sorghum x sudangrass (SxS) hybrid, and Piper sudangrass. Plots had ten rows 30.5 cm apart and were 6.1 m long.

The soil at Lahoma is classified as a fine silty, mixed thermic, Puchic Argiustolls mapped as a Pond Creek silt loam. A soil test taken to a depth of 15 cm revealed 77 and 493 kg ha^{-1} of phosphorous (P) and potassium (K) respectively, and a pH of 5.8. The NO_3-N level of the soil at Lahoma, sampled to a depth of 53 cm was 18 kg N ha^{1}. Both soil tests were conducted on the day of seeding and prior to N application. Both fertilization and seeding were performed on June 14, 1982.

The soil at Perkins is classified as a Teller loam, a fine-

silty, mixed thermic Udic Agriustolls. The soil tests, taken to a depth of 15 cm, revealed a pH of 5.6 and 5.9, P 52 and 91 kg ha^{-1}, and K 193 and 259 kg ha^{-1} for P82 and P83, respectively. The NO_3-N level of the soil at Perkins, sampled to a depth of 53 cm, was 0 and 12 kg ha^{-1} for P82 and P83, respectively. All soil tests were taken on the same day as seeding, prior to fertilization. Fertilization and seeding were done on June 9, 1982 and June 16, 1983 for P82 and P83, respectively.

For nitrate determination, a composite sample of several plants was taken in the afternoon (1400-1600) at weekly intervals from rows not used for yield determination. These were cut 4 cm above ground level, then were cut again 15 cm above the previously excized edge yielding an upper and lower plant sample. Samples were dried in a forced-air oven at a temperature of 60C, for five days, weighed, and ground with a Wiley mill to pass through a 2 mm screen. Samples were analyzed for nitrate using standard Oklahoma State University soil laboratory procedures for plant nitrate (Hanlon and Johnson, 1983). All nitrate ion concentrations are reported as grams of nitrate (NO_3^-) per kg of oven-dried plant tissue. Concentration of total plant NO_3^- was calculated using sample weight and NO_3^- concentration as follows:

$$\text{total plant g } NO_3^- \text{ kg}^{-1} = \frac{(\text{g top})*(\text{top g } NO_3^- \text{ kg}^{-1}) + (\text{g bottom})*(\text{bottom g } NO_3^- \text{ kg}^{-1})}{\text{g top} + \text{g bottom}}$$

Twice during the growing season, at L82 and P82, the same plots were sampled on the following day in the early morning (0700-0900). Since there was no significant effect on plant nitrate due to early morning vs. afternoon sampling, only afternoon data are presented.

On the last sampling dates reported, forage yield was taken with a self propelled Carter harvester, harvesting three rows at a height of 4 cm.

Separate analyses of variance (ANOVA) were performed on all of the data for each location and each year utilizing a 5 x 3, variety x N factorial design. Paired t-tests were calculated at the 1% level of probability for comparison of the lower vs. upper plant nitrate concentrations.

CHAPTER IV

RESULTS AND DISCUSSION

Soil moisture for all three experiments was excellent at planting. However, at both L82 and P83, precipitation amounts dwindled as the summer progressed (Table 1). At L82 only 17 mm of rain fell during the period between the first sampling and the harvest date. At P83 136 mm of precipitation occurred during a three week period following seeding; only 70 mm of rain fell, however, during the period between the first sampling and the harvest date. The plants at P83 were allowed to grow late into the summer in hopes of harvesting following a rain sufficient to stimulate rapid growth ...(which never occurred). Soil moisture was adequate at P82 throughout the early and mid growing season.

N Fertilizer Effect

Increased N rates significantly increased forage nitrate concentrations at both locations and years (Table 2 and Fig. 1, 2, and 3). In each instance, the higher the N rate, the higher the forage nitrate concentration. This relationship persisted throughout the summer growing season for each experiment. Congruency between increased N fertilizer rates and increased forage nitrate concentrations has been reported by many other workers (Clark, Leslie, and Hemken, 1966; Terman, Noggle, and Hunt, 1976; Hanaway and Englehorn, 1958; Moeller and Thurman, 1966).

Table 1. Monthly rainfall totals and deviation from normal at Lahoma and Perkins Research Stations, 1982 and 1983.

Location and year	Item	Mar.	Apr.	May	June	July	Aug.	Sept.
		mm						
L82	Rainfall	58.0	66.0	235.0	80.0	83.0	1.5	17.0
	Deviation†	-13.0	-3.0	151.0	18.0	16.0	-73.0	-69.0
P82	Rainfall	35.0	60.0	371.0	134.0	53.0	8.0	22.0
	Deviation	-21.0	-20.0	242.0	18.0	-34.0	-73.0	-74.0
P83	Rainfall	86.0	54.0	155.0	138.0	0.0	24.0	49.0
	Deviation	30.0	-26.0	26.0	22.0	-87.0	-57.0	-47.0

† = Deviation from the 30- year means.

Table 2. Whole plant nitrate concentration analysis of variance (ANOVA) summary for cultivar types, N fertilizer rates, and date of sampling at two locations and years.

Source	df	L82	P82	P83
Replication (R)	3	*	*	*
Nitrogen (N)	2	**	**	**
Variety (V)	4	**	**	**
N x V	8	NS	NS	*
N_L† x V	4	NS	NS	*
N_Q‡ x V	4	NS	NS	NS
Error a	42	71.4	13.6	19.6
Date (D)	(3)(2)(5)#	NS	**	**
Error b	(9)(9)(15)	79.6	4.6	16.0
N x D	(6)(4)(10)	**	**	**
N_L x D_L	1	**	**	**
N_L x D_{NL}††	(2)(2)(4)	NS	**	**
V x D	(12)(8)(20)	**	**	**
V x D_L	4	**	**	**
V x D_{NL}	(8)(8)(16)	**	*	**
N x V x D	(24)(16)(40)	NS	*	NS
Error c	(126)(126)(210)	28.9	5.2	5.6

*,** Significant at the 0.05 and 0.01 probability levels respectively.
NS not significant
† linear
‡ quadratic
# L82, P82, P83
†† includes quadratic and cubic for L82; quadratic for P82; and quadratic, cubic and quartic for P83.

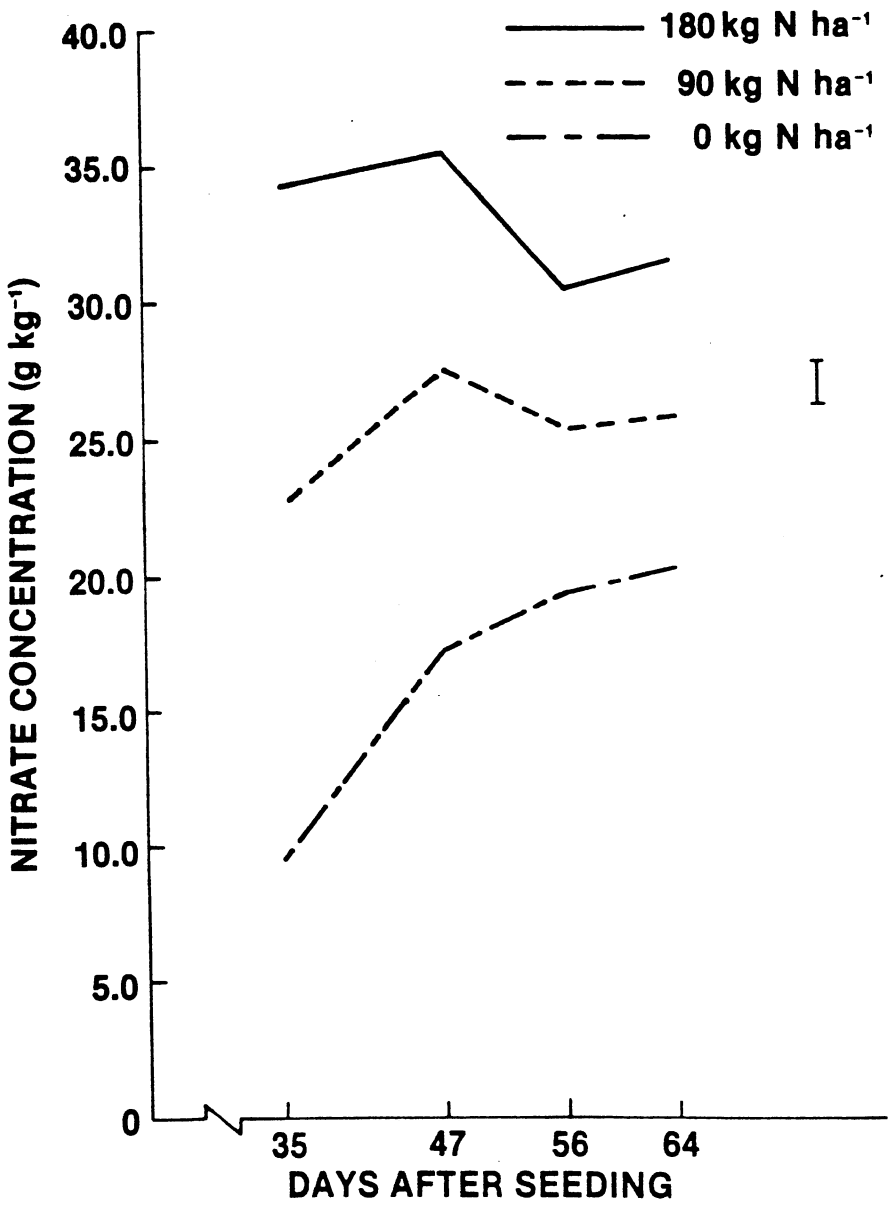

Fig. 1. The effect of N rate on whole plant nitrate concentration at each sampling date for L82. (Each point represents the mean of four replications and five cultivars. Vertical bar represents standard error of difference of two means at the same date).

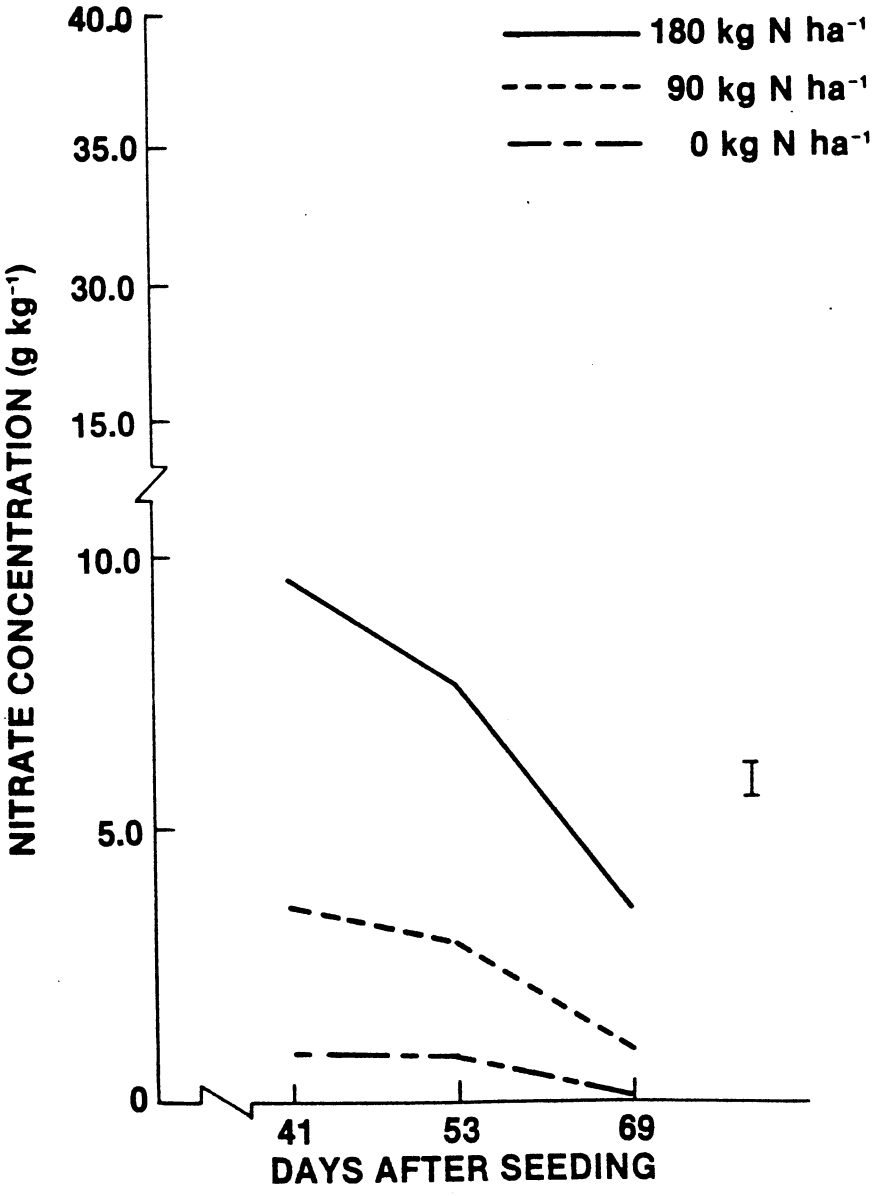

Fig. 2. The effect of N rate on whole plant nitrate concentration at each sampling date for P82. (Each point represents the mean of four replications and five cultivars. Vertical bar represents standard error of difference of two means at the same date).

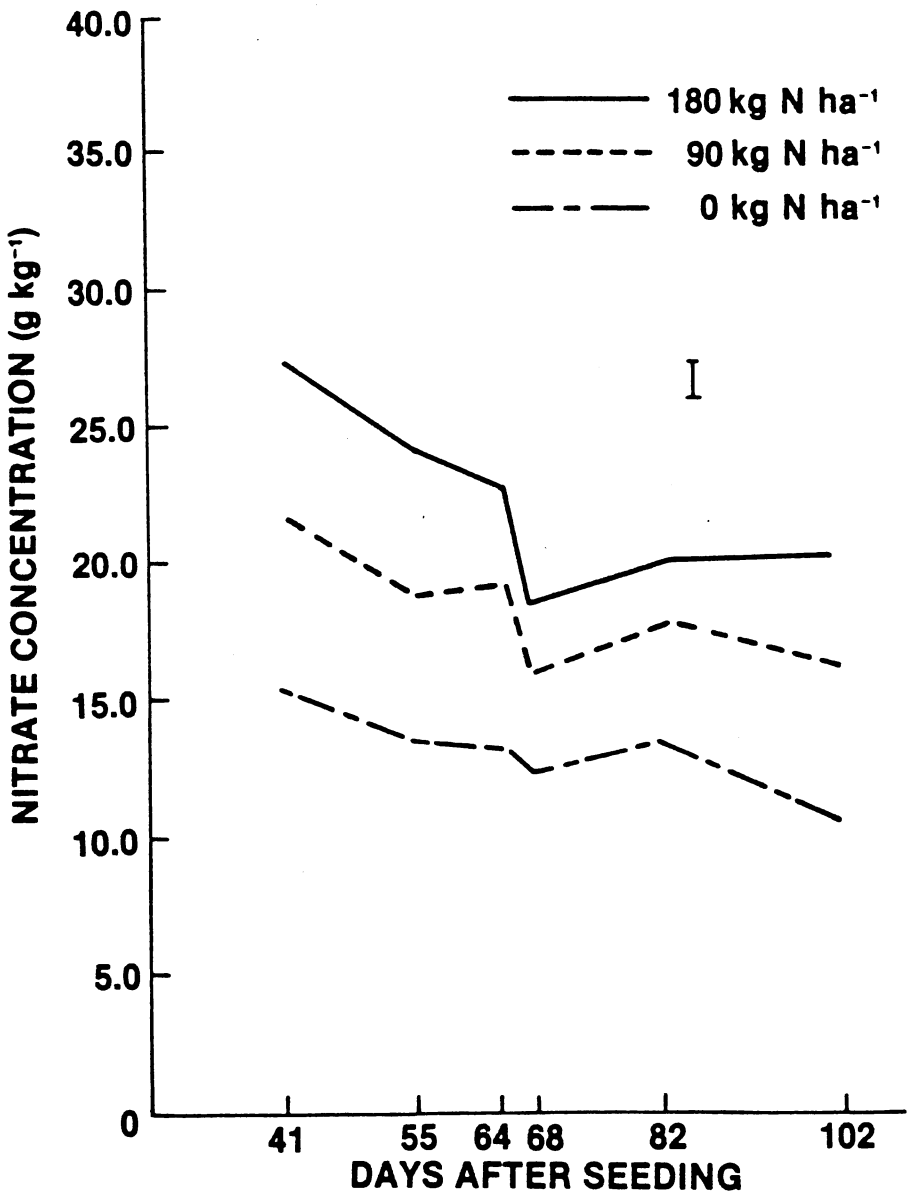

Fig. 3. The effect of N rate on whole plant nitrate concentration at each sampling date for P83. (Each point represents the mean of four replications and five cultivars. Vertical bar represents standard error of difference of two means at the same date).

Only at L82 for the treatments of 0 and 90 kg N ha^{-1} did whole plant nitrate concentrations increase over the sampling period (Fig. 1). Whether any plant nitrate persists to maturity depends to a large degree on a continuing supply of available soil N. The previous years crop at the site for L82 had been mung beans (Phaseolus aureus). Nitorgen in the organic state cannot be accounted for by a simple NO_3-N soil test, and therefore this soil test may have been an insufficient measure of N available for growth. Soil conditions during the early growing season at L82 were ideal for nitrification. Nitrification occurs readily under conditions of warm temperature, adequate oxygen, optimum pH and moisture (Soil Improvement Committee, Calif. Fertilizer Assoc., 1980).

Forage nitrate concentrations increased linearly between N rates for each date, except at L82 (Table 2, Fig. 1, 2, and 3). For L82, forage grown at 0 kg N ha^{-1} had nitrate concentrations that increased throughout the sampling period, from 9.5 to 20.2 g kg^{-1}. However, forage nitrate concentrations decreased between the period of 47 and 56 days after seeding (DAS) for both the 90 and 180 kg N ha^{-1} treatments (Fig. 1). It is not clear what caused this non-uniformity between treatments. As plant growth progressed at P82 and P83, forage nitrate concentrations decreased significantly for all N treatments (Figs. 2 and 3). The observed decrease in plant NO_3^- concentrations at P82 is thought to be due primarily to the adequate soil moisture which was available to the plants through much of the early and mid growing season. Unimpeded plant growth resulting in a dilution of nitrate within the plant (dry matter:nitrate ratio) may also have had an effect on plant nitrate concentrations.

Plants at L82, for each N rate, contained potentially toxic concentrations of nitrate. At L82, the suspected additional soil N probably contributed to the high, unsafe concentrations of plant NO_3^- that were encountered for all treatments. Only those forages grown at 0 kg N ha^{-1} at P83, contained potentially non-toxic concentrations of nitrate, while no plants at P82, at any N rate, contained dangerous levels of NO_3^-.

Species/Variety and Seasonal Growth Stage Effect

At both L82 and P83 the pearl millets accumulated significantly greater concentrations of nitrate throughout the growing season than did the sudangrasses (Fig. 4 and 6). These results are in agreement with those of Clark, Hemken and Vandersall (1965), and Schneider and Clark (1970) who found that pearl millets generally accumulate nitrate to a greater degree than do sudangrasses. Additionally, Smith and Clark (1968) found that pearl millets extract greater amounts of nitrate from the soil than sudangrasses. It appears that the uptake of nitrate from the soil and the reduction of nitrate to nitrite within pearl millet is not well coordinated in that large quantities of nitrate may be extracted from the soil, yet with the onset of moisture stress a cessation of nitrate reductase activity occurs within the plant. This may indicate a more labile nitrate reductase enzyme. At this time the exact interaction between nitrate uptake, assimilation, and the parameters inhibiting or enhancing these processes in pearl millet at the molecular level has not been adequately studied.

For both L82 and P83 on the final sampling date, the pearl millets were significantly higher in nitrate concentration than either the

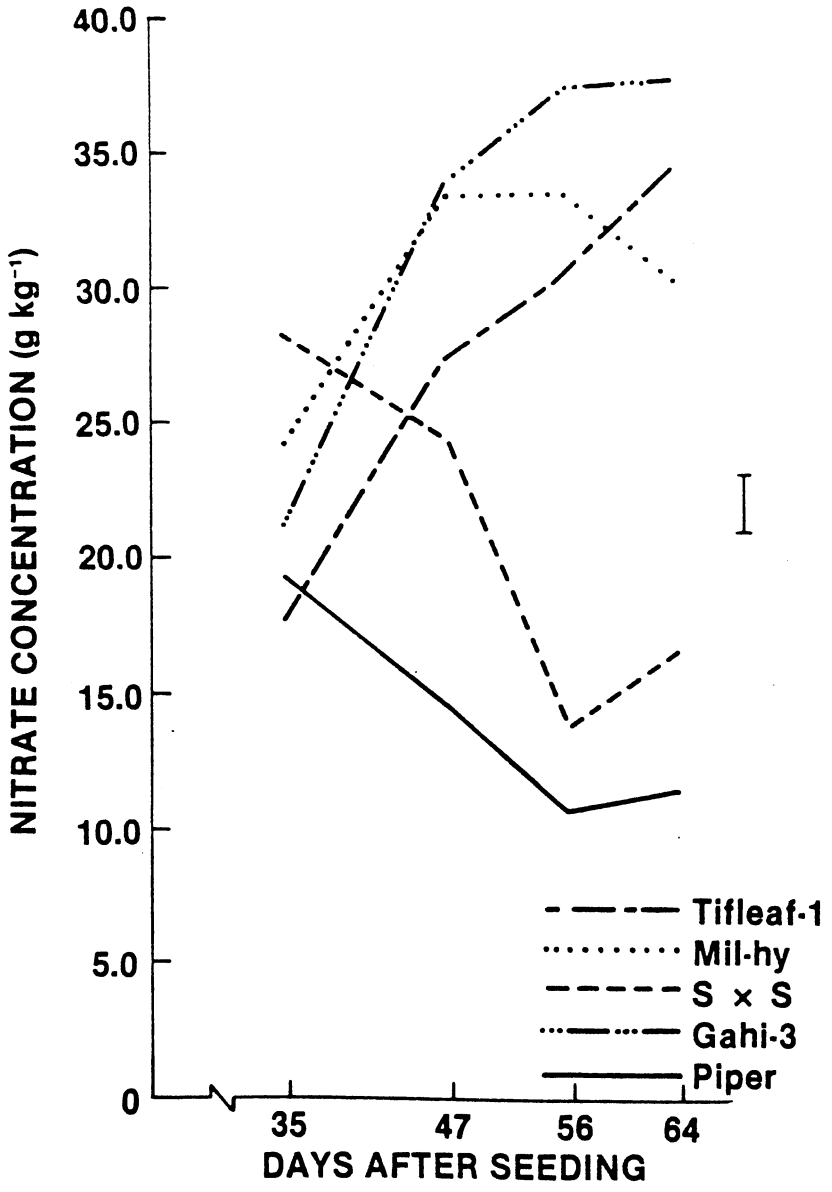

Fig. 4. Whole plant nitrate concentration over 64 days following seeding at L82. Each point represents the mean of four replications and three nitrogen rates. Vertical bar represents the standard error of difference of two means at the same date).

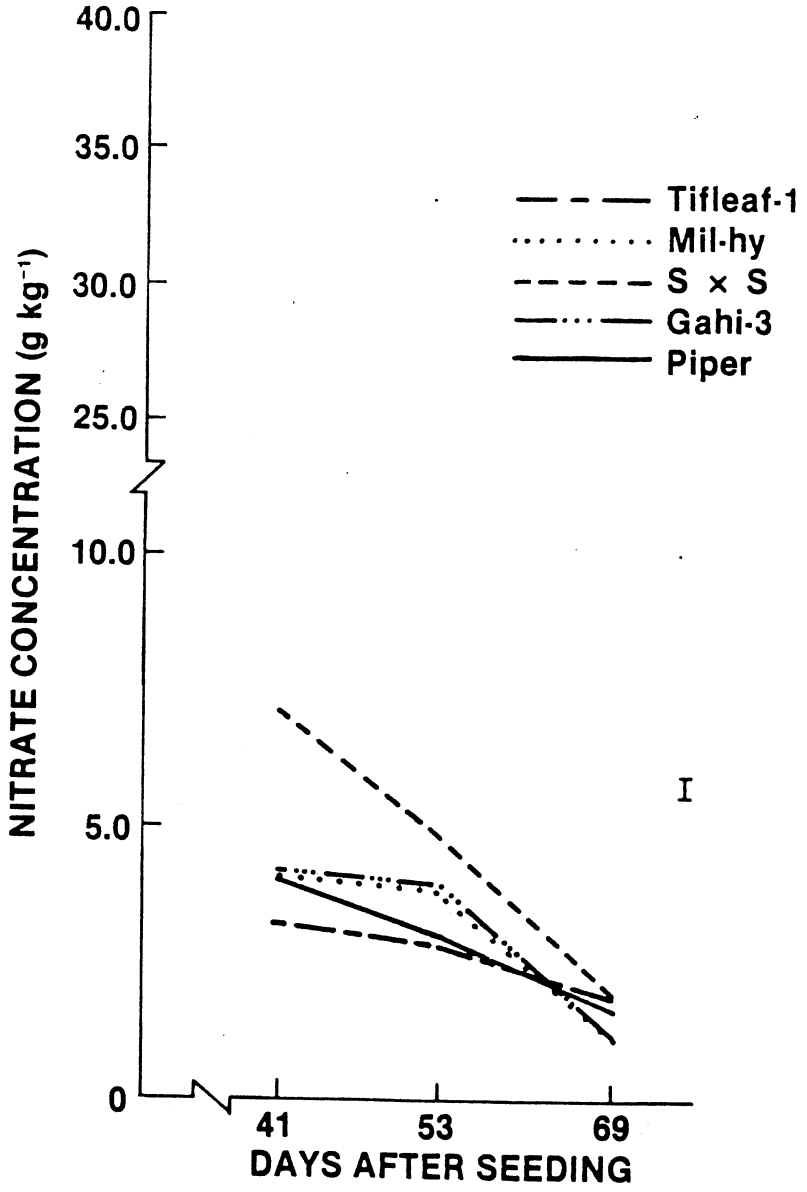

Fig. 5. Whole plant nitrate concentration over 69 days following seeding at P82. (Each point represents the mean of four replications and three nitrogen rates. Vertical bar represents the standard error of difference of two means at the same date).

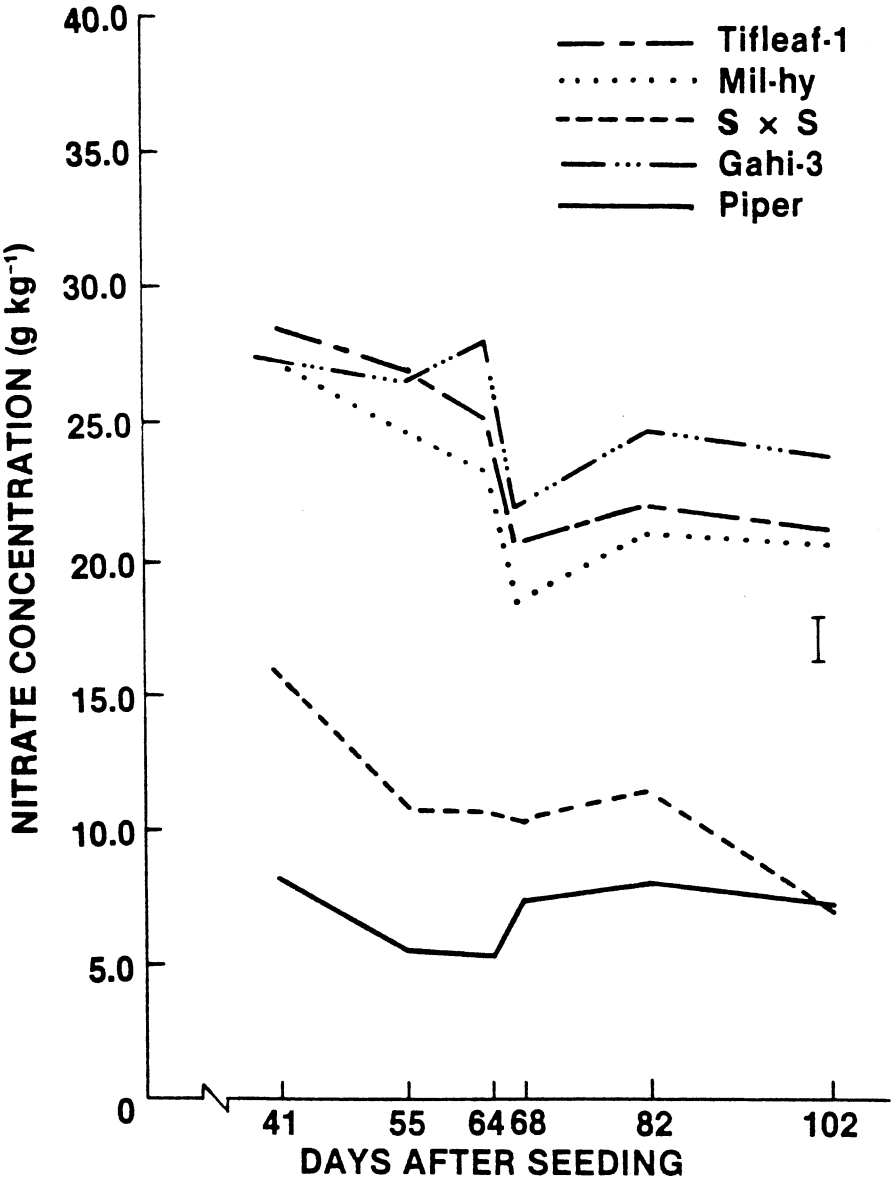

Fig. 6. Whole plant nitrate concentration over 102 days following seeding at P83. (Each point represents the mean of four replications and three nitrogen rates. Vertical bar represents the standard error of difference of two means at the same date).

S x S hybrid or the Piper sudangrass. Two possible factors may have had an influence. The sudangrasses were fully headed in the dough stage while the pearl millets exhibited significant physiological juvenility as confirmed by their lack of terminal spike formation. Therefore, the sorghums were in a more advanced stage of maturity than the pearl millets. It has been observed that nitrate in certain cerals may be high early in the plants life; however as the plant matures, especially during flowering, nitrate levels decline (Gul and Kulp, 1960). The other factor was drought. Only 1.5 mm of precipitation occurred during the month of August at L82. The drought at P83 was more severe in that no precipitation occurred for six weeks from July until mid August. The 70 mm precipitation which occurred during the months of August and September at P83 was in scattered light showers that were inadequate to stimulate rapid growth. The pearl millets that were headed exhibited an inhibition of peduncle emergence, while those that were not headed had severe dessication of terminal leaf margins and tips. Peduncle emergence occurs as a result of cell elongation due to positive turgor pressure in the elongation zone of the plants apex. During periods of moisture stress, plants which have not osmotically adjusted can experience a decrease in turgor pressure (Henson, 1982). Thus, the sorghums may be better adapted to grow under conditions of moisture stress than the pearl millets.

At L82, Gahi-3 contained significantly greater concentrations of nitrate than all other forages at both 56 and 64 DAS. Piper sudangrass contained significantly lower concentrations of nitrate than any of the other four forages except at the initial sampling date (Fig. 4).

At P83, Piper sudangrass contained significantly lower concentrations of nitrate than any other forage, throughout the growing season, until 102 DAS when no significant difference was found between it and the S x S hybird. Gahi-3 pearl millet contained significantly greater concentrations of nitrate than the other millets only at 82 and 102 DAS. At 102 DAS, the final sampling date, only the sudangrasses contained potentially non-toxic concentrations of nitrate (Fig. 6).

Soil moisture at P82 was sufficient throughout the early and mid growing season (Table 1). Correspondingly, concentrations of nitrate in all five forages decreased over time (Fig. 5). Three of the five forages (the two sudangrasses and Mil-hy pearl millet) were 100% headed on the final sampling date. Others have reported that plant nitrate decreased as the plant matures (Hanaway and Englehorn, 1958; Hanaway et al., 1963). On the final sampling date both the Gahi-3 and Tifleaf-1 pearl millets were below 20% heading on this date; yet for all N rates, each of these forages' nitrate concentration decreased over time (data not shown). In this case (P82), a relationship between maturity and a decrease in plant nitrate concentration cannot be made. Clearly other factor(s) for example, sufficient soil moisture, were at work.

The S x S hybrid at P82 had consistently and significantly higher concentrations of nitrate throughout the early growing season than the other four forages (Fig. 5). However, by the time all forage was harvested, no significant difference existed in nitrate concentrations between the forages.

Effect of Cutting Height

For all three studies, at all sampling dates, lower (5-20 cm) plant samples contained significantly greater ($p<.01$) concentrations of nitrate than did the upper plant samples (Table 3). Nitrate concentration in the lower 15 cm samples of harvested forage were from 2.4 to 3.5 times higher than that in the upper part of the forage (data not shown). Our data are in agreement with others who have observed that many plants accumulate nitrate to a greater extent in the stems than the leaves (Terman, Noggle, and Hunt, 1976; Crawford, Kennedy and Johnson, 1961; Baker and Tucker, 1971; Hanaway and Englehorn, 1958). As both whole plant nitrate concentrations and N fertility rates increased, a concomitant increase occurred in the difference between upper and lower plant nitrate concentrations (Fig. 1, 2, 3, and Table 3). Under conditions of moisture stress (L82, P83), Piper sudangrass had the smallest difference between upper and lower plant NO_3^- concentration for each N rate. Ryan, Wedin and Bryan (1972) and McCreery, Hojjati, and Beaty (1966) have reported that as the cutting height of harvested forage was raised, resulting in a lower stem-to-leaf ratio, nitrate concentrations in the harvested forage were reduced. Here we establish that in many cases, raising the cutting height can decrease the concentration of nitrate in harvested forage to a safe, non-toxic or more manageable level.

Time of Day Effect

No significant difference in forage nitrate concentration was found for plants sampled in the morning vs. those sampled in the afternoon. At L82 the grand mean for plant nitrate concentration

sampled in the afternoon was 24.5 g kg^{-1}. This compared to 24.4 g kg^{-1} nitrate detected in the plant samples obtained in the morning. Similar results were found at P82 where the mean plant nitrate concentration for those plants sampled in the afternoon was 4.2 g kg^{-1} while the plant nitrate concentration mean of those plants sampled in the morning was 4.1 g kg^{-1}. It was concluded that no beneficial decrease in plant nitrate could be realized by harvesting in the early morning vs. the afternoon.

Dry Matter Yield

The S x S hybrid yielded significantly greater amounts of DM only at P83 (Table 4). Tifleaf-1 pearl millet produced significantly less dry matter than the other four forages at both L82 and P82. The sudangrasses responded to increased N rates with increased DM production for both P82 and P83 (data not shown).

Summary

Three forage nitrate studies were conducted over a period of two years (1982 and 1983) at two Oklahoma State Agronomy research stations in north-central Oklahoma. Five forages, 'Gahi-3', 'Mil-hy', and 'Tifleaf-1' pearl millets [Pennisetum americanum (L.) Leeke], a sorghum x sudangrass hybrid, and 'Piper' sudangrass [Sorghum bicolor (L.) Moench] were monitored for nitrate concentration at Lahoma, on a Pond Creek silt loam (fine-silty, mixed thermic, Puchic Argiustolls) and at Perkins on a Teller Loam (fine-silty, mixed thermic, Udic Argiustolls). The studies were conducted to relate plant nitrate to nitrogen (N) fertilization at rates of 0, 90 and 180 kg ha^{-1}, seasonal

Table 3. Nitrate concentration differences between lower and upper plant samples for each N rate and experiment over all sampling dates.

Cultivars	kg N ha^{-1} 0	90	180
	g kg^{-1}		
		Lahoma 1982	
Piper	5,8	16.5	29.8
S x S	23.6	32.6	45.2
Gahi-3	20.3	30.7	39.7
Mil-hy	21.0	32.9	39.7
Tifleaf-1	18.5	30.3	45.6
		Perkins 1982	
Piper	0.2	3.3	8.1
S x S	0.9	7.6	13.3
Gahi-3	0.0	2.7	12.4
Mil-hy	0.0	2.4	14.1
Tifleaf-1	0.0	1.5	10.6
		Perkins 1983	
Piper	4.9	8.8	10.5
S x S	9.1	14.2	15.7
Gahi-3	10.9	19.3	26.3
Mil-hy	12.3	17.7	21.5
Tifleaf-1	15.3	21.8	25.7

Table 4. Mean dry matter (DM) yields averaged over N rates for five cultivars at two locations and years.

Cultivar	L82	P82	P83
	----------kg ha^{-1}----------		
S x S	8437	9247	8637
Piper	7389	7154	5940
Gahi-3	6589	8000	6270
Mil-hy	7029	6668	5884
Tifleaf-1	5248	6163	5540
LDS(0.05)	1138	1430	1431

growth stage, and cultivar. Cutting height was evaluated as a technique to reduce nitrate in harvested forage. Nitrate concentration increased in all forages with increased N rates, but decreased with advancing plant age if moisture was adequate for continued growth. The pearl millets accumulated nitrate to a greater degree than the sudangrasses when subjected to extended periods of drought. Among the millets, Gahi-3 accumulated the greatest concentrations of nitrate. Piper sudangrass accumulated the lowest concentrations of nitrate at both L82 and P83. Pearl millets were not as well adapted to growth under conditions of moisture stress as evidenced by their higher concentrations of nitrate and growth inhibition. The lower 15 cm of harvested forage contained significantly greater ($p<.01$) (2.4 - 3.5 x) nitrate concentrations than the upper plant portions. As both whole plant nitrate concentrations and N fertility rates increased, a congruent increase was seen in the difference between upper and lower plant nitrate concentration. Piper sudangrass exhibited the smallest difference in nitrate concentration between upper and lower plant samples at L82 and P83. The S x S hybrid yielded significantly greater amounts of DM than the other forages only at P83.

CHAPTER V

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APPENDIX

Table 5. Percent heading of forages at harvest date for L82, P82, and P83.

Cultivar	L82	P82	P83
	% heading		
Piper	100	100	94
S x S	100	100	99
Gahi-3	05	05	25
Mil-hy	100	100	30
Tifleaf-1	15	15	20

Table 6. Whole plant nitrate concentration of forages sampled at L82 in the afternoon vs. the morning.

Cultivar	Date and Time of Sampling			
	18 July P.M.	19 July A.M.	2 Aug P.M.	3 Aug. A.M.
	g kg^{-1}			
Piper	17.2	19.3	15.5	14.6
S x S	23.9	28.1	25.3	24.3
Gahi-3	21.7	21.1	34.4	33.8
Mil-hy	28.8	24.0	35.9	33.2
Tifleaf-1	18.2	17.8	25.6	27.4

Table 7. Whole plant nitrate concentration of forages sampled at P82 in the afternoon vs. the morning.

Cultivar	Date and Time of Sampling			
	21 July P.M.	22 July A.M.	2 Aug. P.M.	3 Aug. A.M.
	g kg^{-1}			
Piper	4.5	4.0	2.7	3.0
S x S	7.6	7.0	4.0	4.7
Gahi-3	4.8	4.2	3.3	3.9
NukOgt	5.4	4.1	2.6	3.8
Tifleaf-1	4.0	3.3	2.9	2.8

Table 8. Nitrate concentration for lower, upper, and whole plant in response to N rate, for L82, P82, and P83.

Location	N	Lower	Upper	Whole
	kg ha^{-1}	g kg^{-1}		
L82	0	29.2	9.9	16.6
	90	56.3	17.2	25.3
	180	61.8	22.1	32.9
P82	0	0.7	0.4	0.4
	90	5.0	1.5	2.3
	180	15.6	3.9	6.5
P83	0	19.3	8.4	13.0
	90	27.7	11.3	18.3
	180	33.3	13.4	22.1

Table 9. Lower, upper and whole plant nitrate concentration for each cultivar over all N rates at L82, P82, and P83.

Location	Cultivar	Lower	Upper	Whole
		---------- g kg^{-1} ----------		
L82	Piper	28.1	10.8	14.0
	S x S	47.2	12.7	20.6
	Gahi-3	52.6	22.4	32.4
	Mil-hy	53.8	20.2	30.1
	Tifleaf-1	47.3	15.9	27.5
P82	Piper	5.9	2.2	2.7
	S x S	10.6	3.2	4.4
	Gahi-3	6.6	1.5	2.9
	Mil-hy	7.3	1.7	2.9
	Tifleaf-1	5.1	1.0	2.4
P83	Piper	12.9	4.8	6.8
	S x S	19.6	6.6	10.7
	Gahi-3	35.3	15.7	25.2
	Mil-hy	32.6	15.1	22.4
	Tifleaf-1	33.8	12.9	23.8

Table 10. Lower, upper and whole plant nitrate concentration at each sampling date over all N rates and cultivars for L82, P82, and P83.

Location	Date	Lower	Upper	Whole
		---------- g kg^{-1} ----------		
L82	19 July	40.3	14.0	22.1
	2 Aug.	48.7	17.4	26.7
	11 Aug.	48.3	17.8	25.0
	19 Aug.	45.9	18.5	25.9
P82	21 July	9.1	2.7	4.5
	2 Aug.	8.6	2.3	3.7
	18 Aug.	3.5	0.9	1.5
P83	27 July	32.3	13.0	21.3
	10 Aug.	29.2	10.7	18.8
	19 Aug.	27.1	9.5	18.4
	23 Aug.	23.3	11.5	15.6
	7 Sept.	23.4	11.9	17.0
	27 Sept.	25.2	9.5	15.6

Table 11. Cultivar dry matter yields for August and October harvests at P82.

Cultivar	Aug.	Oct.	Total
	kg ha^{-1}		
Piper	7154	1179	8333
S x S	9247	1128	10555
Gahi-3	8000	723	7391
Mil-hy	6668	863	8863
Tifleaf-1	6163	754	6917
LSD (0.05)	1430	596	1532

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