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The Effects of N-Serve on the Availability of Urea and Ureaformaldehyde Nitrogen on *Lolium perenne* L.

Ronald E. Schroll

Eastern Illinois University

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The effects of N-Serve on the availability of urea
and ureaformaldehyde nitrogen on *Lolium perenne* L.
(TITLE)

BY

Ronald E. Schroll

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1980

YEAR

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THE EFFECTS OF N-SERVE ON
THE AVAILABILITY OF UREA AND UREA-FORMALDEHYDE
NITROGEN ON LOLIUM PERENNE L.

BY

RONALD E. SCHROLL

B.S. in Botany
Eastern Illinois University, 1978

ABSTRACT OF A THESIS

Submitted in partial fulfillment of the requirements
for the degree of Masters of Science in Botany at the Graduate School
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ABSTRACT

Laboratory experiments were conducted to determine the effect of "N-Serve" (DOW) on the availability of urea nitrogen and the subsequent greening effect on perennial ryegrass (Lolium perenne L.) seedlings as compared to the availability of nitrogen and subsequent greening from a controlled release ureaformaldehyde fertilizer. A low organic matter sandy loam soil and a high organic matter clay loam soil were used in this study. Soil treatments of urea and ureaformaldehyde fertilizers were applied and incorporated at concentrations of 0, 25, 38, and 50 ppm-N. N-Serve at concentrations of 0.25, 0.50, and 1.0 ppm active ingredient were applied and incorporated with the three highest urea concentrations in all possible combinations. Two hundred grams of soil were placed in styrofoam cups. For each soil, there were six trials corresponding to incubation periods of 0, 2, 3, 4, 5, and 6 weeks. All treatments were replicated three times per each trial. Soil water was maintained at field capacity (by weight) throughout the experiment. All cups were incubated in a room which had an average temperature of 80°F, a light intensity of 400 ft-c at table top, and a 16-hour photo-period. At the end of each incubation period, the soils were sampled for ammonia and nitrate nitrogen. At the same time, twenty seeds of L. perenne L. were planted in the soil. Seedlings were grown for seventeen days and then excised at the soil surface and analyzed for total chlorophyll. Control of nitrification was obtained in both soils by

the use of N-Serve at 0.50 and 1.0 ppm. Maintenance of $\text{NH}_3\text{-N}$ was longest in the clay loam soil at 1.0 ppm N-Serve. The increased persistence of $\text{NH}_3\text{-N}$ in the clay loam soil was attributed to the decrease in volatilization of N-Serve by adsorption to the organic matter and by the bonding of ammonium nitrogen to the clay fraction of the soil. Chlorophyll content of the ryegrass plants was increased in all treatments for both soils during the period of time in which ammonium nitrogen was most prevalent. Urea in conjunction with N-Serve promoted the highest concentrations of chlorophyll by prolonging the availability of ammonium nitrogen. The ammonium released from the ureaformaldehyde treatments nitrified rapidly and did not enhance chlorophyll synthesis in the ryegrass seedlings.

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INTRODUCTION

Turfgrass, like most other plants, requires sixteen essential elements. Nutritional problems which arise with turfgrass usually involve only nitrogen (N), phosphorous (P), and potassium (K). Micro-nutrient problems in turfgrasses are rare: iron deficiency may be caused by high pH or by an excess of phosphorous, while a manganese deficiency may also occur from high pH, excess leaching, or usually because it is in a form which is unavailable to the plant (Beard, 1973).

Nitrogen is usually the most critical element for turfgrass growth. The amount of nitrogen available to the plant will determine the rate of growth, density, disease resistance, tolerance to temperature and moisture stress, and plant color (Staib and Hays, 1980). The need for available nitrogen over the entire growing season and its susceptibility to leaching and denitrification make requirements higher for nitrogen than for other elements.

Synthetic organic nitrogen sources fall into two classes: fast release (urea) and slow or controlled-release (ureaformaldehyde). Fast release fertilizers are primarily water soluble, while controlled-release fertilizers are primarily water insoluble.

Urea is the soluble nitrogen source most frequently applied to home lawns. Urea is characterized by having (a) high water solubility, (b) rapid initial plant response, (c) relatively short residual response, (d) tendency to leach, (e) high foliar burn potential, and (f) a low

cost per unit nitrogen (Beard, 1973). In soil, urea is rapidly converted by the enzyme urease to ammonium carbonate, which is unstable and dissociates to yield free ammonia (Cooke, 1967). In the presence of moisture, ammonia forms ammonium hydroxide which dissociates into ammonium ions (NH_4^+) in the soil solution (Staib and Hays, 1980). Soil bacteria convert the ammonium nitrogen to nitrate (NO_3^-) nitrogen, a process known as nitrification. Nitrate is extremely water soluble, and because of its negative charge is not adsorbed on the negatively charged soil colloids. Thus it is easily leached from the soil-root zone.

The controlled-release ureaformaldehydes are characterized by having (a) medium-low water solubility, (b) an intermediate initial release, (c) long residual response, (d) reduced loss by leaching, (e) low foliar burn potential, and (f) a high cost per unit nitrogen (Beard, 1973). Slow-release nitrogen sources allow a more gradual conversion of fertilizer nitrogen to the nitrate form (Staib and Hays, 1980), thus supplying the plant with a continual source of nitrogen, but at a rate minimizing loss of nitrate nitrogen due to leaching and denitrification.

The use of a nitrification inhibitor to avert the conversion of ammonium to nitrate might well increase the availability and efficiency of urea nitrogen by decreasing loss due to nitrate leaching and denitrification. 2-chloro-6-(trichloromethyl)-pyridine ("N-Serve", registered trademark of DOW Chemical Company) has been found by many researchers to be the most effective nitrification inhibitor available (Bundy and Bremner, 1973; Parr, Carroll, and Smith, 1971).

The purpose of this experiment was to study the effects of the nitrification inhibitor, "N-Serve", on the availability of urea nitrogen and the subsequent greening effect on perennial ryegrass (Lolium perenne L.) seedlings, as compared to the availability of nitrogen and the subsequent greening from a controlled release ureaformaldehyde fertilizer.

LITERATURE REVIEW

Turfs were developed by modern man in order to enhance his environment. Turfs are important in man's activities from the functional, recreational, and ornamental standpoint. Functional aspects of turfgrass range from controlling wind and water erosion of soil, to use in climate control by reducing glare, noise, heat buildup, and dust stabilization. Many recreational activities (for instance, baseball, golf, and football) utilize turf. Because of man's life style and increasing urbanization, turf provides aesthetic value by making cities, homes, and businesses more pleasurable.

Turfgrasses, having a temperature optimum of 60° to 75°F, are referred to as cool season turfgrasses. The majority of the cool season turfgrasses belong to the following genera: Poa (bluegrass), Agrostis (bentgrass and redtop), Festuca (the fescues), and Lolium (ryegrass). Those species having a temperature optimum of 80° to 95°F are referred to as warm season turfgrasses. Some of the members of this group are the genera Cynodon (bermudagrass), Zoysia, Stenotaphrum (St. Augustine-grass), and Axonopus (carpetgrass).

The cool season ryegrass, Lolium perenne L., was used in this study. L. perenne is the ryegrass species most widely used as a turfgrass, and is thought to be one of the earliest cultivated grasses. Beard (1973) describes the species as: "vernation folded; sheaths somewhat compressed, glabrous, loose, lower sheaths reddish at base,

split with overlapping margins; ligule membranous, 0.5-1.5 mm long, truncate; collar conspicuous, narrow to medium broad, divided, glabrous; auricles small to moderate in size, claw-like, soft; blades flat, 2-5 mm wide, glabrous, glossy below, dull with prominent veins above, keeled, acute apex, margins usually scabrous; stems compressed, erect to somewhat decumbent at base, tufted; inflorescence erect, spike-like, long, narrow, flat spikes with awnless spikelets positioned edgewise to the rachis."

Perennial ryegrass is most often utilized where rapid establishment and soil stabilization are desired, such as slopes which have a high potential for erosion, and when the probability of successful establishment of the turf is low because of drought or time of year. Perennial ryegrass is usually used in a seed mixture; for instance, with Kentucky bluegrass at a rate of 20 to 25 percent of the mixture. A higher ryegrass content of the mixture may result in excessive competition with the desired turfgrass species (Beard, 1973).

Nitrogen is a vital constituent of the chlorophyll molecule, amino acids and proteins, and nucleic acids. Nitrogen nutrition affects turfgrass shoot growth, root growth, shoot density, color, disease resistance, and heat, cold, and drought hardiness. The color of the turfgrass is directly correlated with the level of nitrogen.

Plants absorb nitrogen in both inorganic and organic forms. The most effective nitrogen sources for most plants are the inorganic ions nitrate (NO_3^-) and ammonium (NH_4^+). Nitrate is the most abundant form of soil nitrogen available to the plant. Ammonium is sometimes relatively abundant; for example, where nitrogen fixation occurs and under

wet, anaerobic conditions. Ammonium is toxic, however, and large quantities may put a strain on the carbohydrate metabolism of the plant in providing carbon skeletons for its detoxification. Plants which grow better on ammonium include many acid plants such as Rumex, which is able to detoxify ammonium by forming ammonium salts of organic acids. The so-called "amide plants", such as beet, spinach, and squash, are able to form the amides glutamine and asparagine from their corresponding dicarboxylic amino acids. Other plants which utilize ammonium are potato, pineapple, Chenopodium album (lamb's quarter), and young cereals such as rice, wheat, corn, oats, and rye. As cereals age, their ability to use nitrate increases so that, when mature, they may respond better to nitrate than the ammonium source of nitrogen. This may relate to the abundance of carbohydrates and reducing power in the mature plant.

Some plants supplied with both ammonium and nitrate in liquid nutrient solution will absorb either ion depending on the pH. If the nutrient solution is basic, the plant will absorb ammonium, and eliminate H^+ by exchange, which thus lowers the pH by forming nitric acid with the nitrate left behind. However, if the pH is acidic, the plant will absorb nitrate, and eliminate OH^- by exchange, which raises the pH by forming ammonium hydroxide. It is concluded by many workers that plants utilize ammonium under slightly alkaline conditions, while nitrates are absorbed from slightly acidic conditions.

Organic nitrogen does not comprise a major source of nitrogen for plants. Organic nitrogen in the soil becomes available to the plant due to the death and decay of microbial, plant, and animal matter into

amino acids. It has been concluded that most plants can absorb amino acids to some extent, but they are usually less effective nitrogen sources than are the inorganic forms. The absorption of more complex organic compounds, such as pyrimidines, purines, and protein has been demonstrated. However, the utilization of these compounds is minimal and is insignificant to plant nutrition.

The first organic nitrogen compound to be studied as a nitrogen source was probably urea. It was discovered in the 1940's that urea could be absorbed directly through the leaves as well as the roots of plants. Urea may be incorporated directly by condensation with ornithine to form arginine, or it may be converted directly to carbamyl phosphate, a precursor of pyrimidines and citrulline.

The nitrogen fertility requirement for L. perenne ranges from 0.4-1.0 lb. per 1000 sq. ft. per growing month. Higher fertility levels decrease the tolerance of ryegrass to environmental stress, run the risk of foliar burn, and force top growth at the expense of root development (Beard, 1973). Root growth of turfgrass practically ceases when luxury consumption of nitrogen occurs. When application of nitrogen leads to rapid growth, the grass must still be mowed to the desired height, but removal of more than 40 percent of the top grass stops root growth (Staib and Hays, 1980).

A nitrogen deficient plant is usually recognized by a yellowing or chlorosis. With grasses, the lower leaves usually "fire" or turn brown, beginning at the leaf tip and progressing along the midrib until the entire leaf is dead. The tendency of the younger leaves to remain green while the older leaves yellow or die is indicative

of nitrogen mobility in the plant. When the roots are unable to absorb sufficient nitrogen for plant growth, nitrogen compounds in the older plant parts will undergo autolysis. The protein nitrogen is converted to a translocatable form, translocated to the active meristematic regions, and is reused in the synthesis of new protoplasm (Tisdale and Nelson, 1965).

The role of nitrogen fertilizer in plant productivity has been a major concern of agronomists and home gardeners for many years. World use of nitrogen represents some 45-50 percent of the total tonnage of plant nutrients used. In the United States, nitrogen is applied to most croplands, gardens, and lawns as anhydrous ammonia, aqua ammonia, urea, or other nitrogen solutions. These nitrogen sources amounted to approximately 8.4 million tons of total nitrogen applied in 1970 (Norris, 1972).

Growing economic and environmental concerns during the past few years have created much interest in nitrogen fertilizers. Nitrogen fertilizers are subject to many chemical and biochemical changes after application to the soil. These changes often result in significant losses of nitrogen. The bacteria, Nitrosomonas and Nitrobacter, the most common nitrifying organisms in the soil, oxidize the ammonium ion to nitrite (NO_2) and nitrate, respectively.

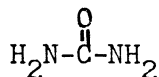
The anion forms, nitrite and nitrate, are not held electrostatically in soil like ammonium because of the respective negative and positive charges. The anions, therefore, are easily leached out of the root zone with rain or irrigation water. Leaching and runoff losses not only reduce the amount of nitrogen available to crops, but

also increase the potential for pollution of surface and ground water (Sander and Barker, 1978). Other losses of the anion forms of nitrogen result from biological denitrification by various heterotrophic bacteria, such as Pseudomonas and Micrococcus, which convert nitrite and nitrate to the gaseous forms of nitrogen: N_2 , N_2O , and NO . Plants grown in high external concentrations of nitrate may accumulate high levels of the ion in edible portions of the plant. Consumption by humans of high nitrate levels in fresh vegetables is considered potentially hazardous.

The cation, ammonium, is also lost from the soil-root zone. This primarily occurs from the volatilization of ammonia from improper application of anhydrous or aqua ammonia and from surface application of urea and nitrogen sources containing ammonium. It should be emphasized that, except for ammonia volatilization, ammoniacal nitrogen fertilizers are subject to loss only after nitrification to nitrite or nitrate (Parr, Carroll, and Smith, 1971).

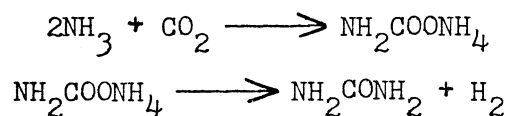
The two nitrogen fertilizers used in this study were urea and ureaformaldehyde. Both are classified as synthetic organic nitrogen sources, but the two vary in their rate of nitrogen release and, thus, their differing effects of plants.

Urea, or carbamide as it is sometimes called, is the most common fertilizer used on turf. It is a nonionic fertilizer, with the molecular formula $CO(NH_2)_2$, and a molecular structure as follows:



Urea has a molecular weight of 60.06 g, a melting point of 133-135°C, a density of 1.335, and is composed of 46.6 percent nitrogen.

Urea was introduced commercially in the United States in 1935. It is prepared by reacting anhydrous ammonia and carbon dioxide gas under very high pressure in the presence of a suitable catalyst. The reactions involved are represented in the following equations:



In soil, urea is converted to ammonium carbonate by hydrolysis reaction in the presence of the enzyme urease. This conversion is indicated by the following equation:



Ammonium carbonate is unstable, and breaks down to form ammonia (NH_3). Under alkaline conditions or in the presence of sufficient moisture, ammonia forms ammonium hydroxide (NH_4OH), which disassociates to free ammonium ions in the soil solution. In soil temperatures above 60°F soil bacteria convert the ammonium to nitrate. This conversion may be complete in two weeks at 75°F.

The immediate effect of urea on the soil reaction is alkaline by the formation of ammonium carbonate. The nitrification of the ammonium ion, however, results in the formation of an acid residue (Tisdale and Nelson, 1955).

Laboratory and field work with urea on various crop plants has shown urea to often be inefficient in promoting growth when compared

to other nitrogen sources. Nitrogen fertilization of grassland with urea has shown this inefficiency. Templeman (1961) showed urea to be slightly less effective than "Nitro-Chalk" (ammonium nitrate-limestone mixture). In most experiments, urea and "Nitro-Chalk" did not differ significantly in yields, but urea was noticeably less efficient with larger applications. In eight out of ten experiments, Devine and Holmes (1963) found urea gave less yield than ammonium nitrate. In the same study, ammonium sulphate proved to be at least as efficient as ammonium nitrate, but urea was no more than three-quarters as efficient. Dilz and Van Burg (1963) found similar results in that urea was usually less efficient than ammonium nitrate-limestone fertilizer. They attributed inefficiency to ammonia loss by volatilization, since losses were less when rain fell immediately after urea application.

Court et al., (1963) report that low rates of urea and ammonium nitrate gave similar yields with maize, but at higher rates urea yielded less. Response to urea was positively correlated with ammonia absorption capacity and moisture content.

Gasser (1965) found nitrogen losses from surface-applied urea varied from 2-13 percent of the applied nitrogen. Losses were greater for sandy soils and less from clays, and were decreased by incorporation of the urea with the soil.

It is concluded from the grassland work that, on the average, 100 lb. of urea nitrogen may be expected to give the same yield as 80 lb. of nitrogen supplied as ammonium nitrate. Often urea and ammonium nitrate will give similar yields, but frequently urea will be less

efficient (Cooke, 1967). The most pronounced inferiority of urea was with surface application. When urea is correctly applied, increased efficiency of the fertilizer is often obtained.

In an early experiment by Widdowson and Penny (1960), damage to germinating cereals occurred from combine-drilled urea application. Widdowson, Penny, and Williams (1964) overcame this effect by placing urea in side-bands. When urea was applied in a band one inch to the side of the seed at a rate of 78 lb. N per acre, 112 lb. more barley grain were obtained than with an equivalent amount of ammonium sulphate.

Side-dressing of the urea limits both damage to germinating seedlings and also losses of ammonia nitrogen by volatilization to the air. The experiments of Widdowson, Penny, and Williams (1960 & 1964) show how a fertilizer, when applied by ordinary methods, is inefficient, but may be as or more efficient than other fertilizers when correctly applied. This observation is supported by the work of Narain and Datta (1974). In their pot study, 150 kg N/ha each of ammonium sulphate, urea, and ammonium nitrate were applied by incorporating each fertilizer with the soil. Ammonium sulphate and urea gave yields of rice superior to the ammonium nitrate. In the same study, all three fertilizers were equally efficient for wheat yield. The increased efficiency of urea was probably due to low loss of nitrogen from ammonia volatilization, since the fertilizer was incorporated in the soil,

Urea fertilizer has been found to improve protein quantity and quality in many plants. In a rangeland dominated by the undesirable annual-grass, Themeda quadrivalis (L.) O. Kuntze, and the desirable perennial spear-grass, Heteropogon contortus (L.) P. Beauv. ex Roem.

& Schult., Namdeo and Dube (1971) used the preplant herbicide dalapon in combination with urea fertilization. In conjunction, the two enhanced the protein content of the perennial grasses. Urea alone gave a 50.5 percent enhancement of the natural regrowth of the perennial grass.

The quantity and quality of wheat protein was enhanced by urea in experiments of Srivastava et al., (1971). At high levels of urea (60 & 80 kg/ha), both foliar and soil treatments doubled the yield of "S 227" wheat. At 20 kg/ha, the foliar application increased wheat yield by 66 percent, whereas the soil treatment increased yield by 31 percent. Soil treatment enhanced protein content 11 percent when urea was applied at 60 and 80 kg/ha. Foliar applications at the rates of 40 and 80 kg/ha, enhanced protein content 11 and 20 percent respectively. Quality of protein was significantly increased by both foliar and soil treatments. Foliar application only slightly increased the concentration of lysine at 40 kg/ha, while the soil treatment enhanced lysine content 30 percent. Tryptophan levels were increased by both soil and foliar methods. The maximum increase of 42 percent was obtained with a foliar treatment of 80 kg/ha. The workers showed that by selection of the level of nitrogen and mode of application, a high yield with a slight increase in both protein and the essential limiting amino acids can be obtained using urea fertilizer on "S 227" wheat.

The growing importance of urea as a nitrogen fertilizer emphasizes the need to overcome the problems encountered in the use of this fertilizer. The problems, previously cited, include damage to germinating seedlings and young plants, nitrite toxicity, and gaseous loss of urea

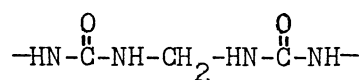
nitrogen (Bundy and Bremner, 1973). These problems result from the rapid hydrolysis of the chemical to ammonium carbonate in most soils through urease activity and the concomitant rise in pH and liberation of ammonia.

Two approaches have been taken in trying to overcome these problems. One approach is to find compounds that will inhibit soil urease activity when applied to soils in conjunction with fertilizer urea. Bremner and Douglas (1971) evaluated more than 100 compounds as inhibitors of urease activity in soils. Their results indicated that, of the compounds thus far tested as urease inhibitors, 2,5-dimethyl-p-benzoquinone, 2,5-dichloro-p-benzoquinone, and 2,6-dichloro-p-benzoquinone are the most effective for retardation of urea decomposition in soils and reduction of the problems caused by the usual rapid hydrolysis of urea by soil urease. Bundy and Bremner (1973) studied the influence of different substituted groups on the effectiveness of substituted p-benzoquinones as inhibitors of soil urease activity. Their work, in consideration of Bremner and Douglas (1971), indicates that the compounds 2,3-dimethyl, 2,5-dimethyl, and 2,6-dimethyl-p-benzoquinone are likely to prove the most effective for inhibition of urease.

The second approach to increasing the efficiency of urea is to encapsulate or coat the urea with elemental sulphur. Nitrogen is released from sulphur coated urea (SCU) by actual diffusion of urea through pinhole openings in the coating. The thickness of the coating, plus imperfections in the surface, determine the rate of nitrogen release (Boots Hercules Agrochemical Co., b). This reduces leaching and runoff losses and slows chemical and biological immobilization

of nitrogen in soils, and nitrification and nitrogen loss through ammonia volatilization and denitrification. It should also supply nitrogen for plant use at a more controlled rate and over a longer period of time (Rindt, Blouin, and Getsinger, 1968). Dalal and Prasad (1975) found sulphur coated urea to increase efficiency when both SCU and urea were applied as surface applicants on a calcareous soil. SCU fertilization of sugarcane gave higher yields of cane and sucrose than urea. Subsurface application of urea and SCU showed an increase in urea efficiency, probably due to a lesser loss of ammonia from volatilization, and no significant effect on the efficiency of SCU.

Ureaformaldehyde (UF) is an organic nitrogen fertilizer which is prepared by reacting urea with formaldehyde under controlled conditions and in prescribed proportions. The products of this reaction are a series of low-solubility and water-insoluble carbon-nitrogen units known as methyleneureas. The general structure of methyleneurea is represented as follows:



The reaction mixture contains 38 percent total nitrogen, of which 11 percent (29 percent of the total nitrogen) is water soluble and 27 percent (71 percent of the total nitrogen) which is water-insoluble. The cold water soluble fraction consists of short-chain polymers which are easily converted by soil organisms to ammonium and nitrate forms of nitrogen. It is desirable to have at least 25 percent of the total nitrogen in the water soluble fraction (WSF) (Beard, 1973). The

water-insoluble fraction (WIN) contains intermediate molecular weight polymers which are soluble in hot water (HWS) and longer chains which are insoluble in hot water (HWIN) (table 1).

As the solubility decreases, each succeeding fraction is more resistant to microbial decomposition, but nevertheless is eventually converted to available nitrogen. The cold-water and hot-water soluble fractions are released over a period of weeks, but the HWIN fraction is slower and may release some of its nitrogen in the following growing season (Staib and Hays, 1980).

Performance of ureaformaldehyde fertilizer is affected by several physical and chemical factors. These factors directly affect solubility and therefore nitrogen availability. The physical and chemical properties important in the performance of ureaform fertilizers are closely related to solubility characteristics; namely, (1) particle size, which affects the rate of solubilization and hence the rate of nitrogen release, and (2) molecular weight distribution, which correlates directly with solubility and with the rate of biological breakdown to available nitrogen (Hays).

Nitrogen availability from fertilizers other than nitrate is generally decreased at lower temperatures because of slower ammonification and nitrification reactions. Depending on the relative effects of temperature on the ammonification and nitrification reactions, the proportionate decrease in overall nitrification rate of ureaforms may be more or less than the effect on ammonia fertilizers. Nitrogen release is rapid at soil temperatures of 90°F and is very slow below 50°F. At cooler temperatures, the cold water soluble fraction (CWS)

is affected to a smaller extent. Thus, a modified ureaform with a greater CWS portion would be better for cool climates.

The effects of low temperature on the biological reactions affecting nitrogen release from ureaforms are real, but the low temperatures usually come early in the season when rapid plant growth generally does not occur. Therefore, the effect of temperature on the performance of ureaformaldehyde is probably minimal (Hays).

The activity index (AI) of ureaforms is an empirical value that attempts to characterize the rate at which residual nitrogen becomes available to the plant. It is calculated as follows:

$$AI = \frac{WIN-HWIN}{WIN} \times 100 = \frac{II}{II+III} \times 100$$

The AI is only an empirical figure and simply shows the amount (percent) of the CWIN portion that goes into hot water. Its utility rests on the assumption that, if the value is high enough (40 percent), the remainder will not be too highly condensed to become available over an extended period of time. Ureaform fertilizers are made by various processes and may be made up of differing kinds and distribution of molecular species. Therefore, direct comparison of AI values is valid only if the products are made by the same process.

The AI value gives information only about the relative size of fractions II and III, but it tells nothing about the nature of fractions II and III other than their solubility in hot water. The size of these fractions does not reveal how they will contribute to fertilizer properties. For example; trimethylenetetraurea ($\text{NH}_2\text{CO}(\text{NHCH}_2\text{-NHCO})_3\text{NH}_2$),

is insoluble in cold water and soluble in hot water. This polymer would be found almost entirely in fraction II. Recent work shows its performance to be little different from that of a cold water soluble source. If fraction II were completely made up of trimethylene-tetraurea and fraction III of an insoluble, highly crosslinked polymer with totally unavailable nitrogen, the ureaformaldehyde composition would meet AI specifications but, in field application, would be of little use as a slow-release fertilizer. This is an extreme case, and is unlikely to occur in the production of ureaforms. However, variation in processes could lead to variations in molecular weight distribution and in solubility-release relationships. These cannot be predicted from the AI, and only actual field experience or laboratory nitrification curves that are obtained under conditions simulating the field can accurately predict the performance of ureaforms (Boots Hercules Agrochemicals Co., a).

The performance of ureaforms on various crops has often shown ureaforms to be inferior to other forms of nitrogen fertilizers. Wilcox (1973) fertilized muskmelon plants with ammonium nitrate, UF, and SCU in a sandy soil. Muskmelon yield was greatest with ammonium nitrate at 80-90 kg/ha. The increased yields were associated with larger vines that produced more fruits. The slow-release fertilizers were believed to be inferior because they did not establish a high enough nitrogen concentration in the soil at the beginning of the season for optimum vine development to promote optimum total fruit yield.

Alessi and Power (1973) studied the effects of various nitrogen sources and rates on Triticum aestivum L. and Hordeum vulgare L.

The plants were fertilized with ammonium nitrate, ammonium sulphate, calcium nitrate, Uramite (ureaform), and Ureaform at rates of 0, 34, and 68 kg N/ha. All the nitrogen sources increased growth. Nitrogen uptake (determined by plant tops) was greatest with ammonium and nitrate sources at 68 kg/ha. The recovery of nitrogen was also lower with the ureaformaldehydes at 44 percent, as compared to 78 percent from the ammonium and nitrate sources. The workers concluded, that over this long term study (8 years), the results indicate ammonium and nitrate fertilizers are superior to the ureaformaldehydes.

Power (1979) reports similar findings working with a native mixed prairie composed of Agropyron smithii (western wheatgrass), Stipa viridula (green needlegrass), Bouteloua gracilis (Blue grana), Carex (dryland sedge), Poa (bluegrass), and Fescuta octaflora (six-weeks fescue). Fertilizer treatments were 0, 56, 225, and 900 kg N/ha of SCU, UF, and ammonium nitrate. Dry matter production from all rates of nitrogen application was greatest for ammonium nitrate and least for ureaformaldehyde. The researchers assumed that about 100 kg N were immobilized in grass roots, and that fertilizer N not accounted for in tops, roots, and soil inorganic N forms estimates gaseous loss. They concluded gaseous loss from ammonium nitrate to be 10 percent and 60 percent for ureaformaldehyde. This 60 percent gaseous loss for UF is most probably the combination of immobilized organic nitrogen with a lesser extent attributed to gaseous loss.

Wilkinson (1977) has shown similar results with ureaforms. In his study, treatment response was measured by turf quality ratings, clipping weight, and nitrogen uptake by Merion Kentucky Bluegrass.

At comparable rates (2 kg N/are), UF produced lower turf quality and clipping weights than ammonium nitrate.

The previous workers have evaluated the nitrogen fertilizer performance by determining the recovery of nitrogen in the crop and by equating the result with fertilizer efficiency. This method is not adequate since nitrogen recovery from a crop, grown under the best field conditions, is not likely to be greater than 50 to 70 percent. Ureaform evaluation by this method often shows it to be as low as one-half that of soluble fertilizers, and it is interpreted to be less efficient (Hays).

Brown (1964, cited from Hays), using the N^{15} tracer technique in recovery experiments of nitrogen, has given more accurate recovery data for nitrogen fertilizers (table 2). The same total recovery was obtained from UF as for ammonium nitrate. The results indicate that part of the fertilizer nitrogen is incorporated into the soil organic matter. Ammonium nitrogen from $N^*H_4NO_3$ as well as from UF are utilized in the organic matter in preference to N^*O_3 . When allowance is made for this "carry over" nitrogen, there may be no difference in recovery from various nitrogen fertilizers (Hays).

Kaempffe (1966, cited from Hays) reports similar results to those of Brown (1964, cited from Hays). His results (table 3) show a high percentage of ureaform recovery and, from them, he has drawn the following conclusion: "When the nitrogen supply to the roots is high, clipping growth is greatly stimulated and the bulk of the nitrogen is recovered in the clippings. When the nitrogen supply is low, growth is greatly suppressed and very little nitrogen is removed in the scanty

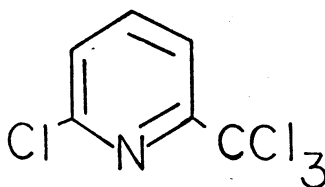
clippings, but the nitrogen supply serves to sustain the density of the crown and stubble--that grass below the clipping height and above the roots. The amount of recovered nitrogen associated with the roots tends to remain relatively constant but may decline with severe N deficiency. Therefore, correlation between nitrogen mineralization in incubation studies, and the ability of grass to absorb this mineralized N, is realized for whole UF only if the whole plant is analyzed for total nitrogen. Clipping accumulation expresses rapid availability but does not accurately reflect the long-term recovery of the mineralized insoluble UF condensates".

Evaluation of ureaform fertilizers on the basis of nitrogen recoveries is valid only when the plant and soil are analyzed for total nitrogen. When this is done, ureaforms tend to be as efficient as the soluble nitrogen fertilizers, such as ammonium nitrate.

Current approaches to improving the efficiency of ammoniacal fertilizers involve the inhibition of Nitrosomonas, the bacterium responsible for converting ammonium to nitrate. N-Serve and Potassium azide (KN_3) have been found to be the most effective and efficient chemicals to inhibit nitrification.

Potassium azide undergoes dissolution in the soil and may be hydrolyzed to hydrazoic acid (HN_3) or ionized to N_3^- , both of which are nonselective and effect all microorganisms in the soil (Cochran, Papendick, and Woody, 1973; Parr, Carroll, and Smith, 1971; Kapusta and Varsa, 1972). HN_3 and N_3^- are subject to chemical decomposition and may leave little or no residue.

N-Serve was the nitrification inhibitor used in this study. The active ingredient of N-Serve Nitrogen Stabilizer has the chemical designation 2-chloro-6-(trichloromethyl)-pyridine. This chemical is also known by the synonym DOWCO 163 and the common name nitrapyrin. Technical grade nitrapyrin has a molecular formula $C_6H_3Cl_4$, a molecular weight of 230.9 g, and a chemical structure as follows:



Nitrapyrin has the following physical properties: a white crystalline solid with a mild sweetish odor, a melting point of 62-63°C, a boiling point of 101 C at 1 mm Hg, and an autoignition temperature of more than 550°C.

The chemical has a low water solubility and excellent solubility in acetone, xylene, methylene chloride, and anhydrous ammonia (table 4). Nitrapyrin is characterized by Goring (1962a) as being a slightly volatile compound which permits it to move through the soil profile (table 5a). Briggs (1975) characterizes nitrapyrin as being an extremely volatile compound as compared with the herbicide trifluoralin (table 5b). Nitrapyrin has a vapor pressure nearly ten times that of trifluoralin; the latter must be incorporated immediately after broadcast or it is ineffective due to volatility. Briggs found that 80 percent of the nitrapyrin, applied as broadcast, volatilized overnight in the laboratory, and that, in a similar experiment conducted in the open air, only 8 percent of the inhibitor remained after three days.

Redeman et al. (1964) reports the concentration of nitrapyrin in soil decreases exponentially with time as a result of volatilization and hydrolysis. Soil texture and percent organic matter play an important role in volatilization of the inhibitor. In general, losses from soil are reduced with light textured soil and high organic matter (Goring, 1962a,b; Hendrickson, Walsh, and Keeney, 1978; Frye et al., 1980). Hendrickson et al. (1978) found nitrapyrin rapidly volatilized and hydrolyzed in a sandy soil. The chemical's short persistence was correlated to the low organic matter and the high porosity of the sandy soil. Briggs (1975) reports the $L_{1/2}$ (half-life) of nitrapyrin is 28 days in a low organic soil, and a $L_{1/2}$ of 50 days in a high organic soil. Although nitrapyrin loss is reduced by high organic matter, the organic matter tends to increase nitrapyrin adsorption and thereby decrease its activity (Hendrickson, Walsh, and Keeney, 1978). Lewis and Stefanson (1975) report control of nitrification was best in near neutral soils with a low C:N ratio, and that effectiveness and period of inhibition by N-Serve was reduced by high carbon contents. Goring (1962a) reports the chemical is highly adsorbed to the organic fraction, but not appreciably to the clay fraction in most soils.

Herlihy and Quirke (1975) studied the persistence of nitrapyrin in three soils: a loamy sand, a coarse sandy loam, and loam. At 10°C the $L_{1/2}$ of nitrapyrin varied from 42-77 days, and at 20°C the $L_{1/2}$ varied 9-16 days. Q_{10} values for the three soils were 5.1, 4.8, and 2.7 respectively, with the coarse textured soils having the highest values. The coarse soil Q_{10} values are outside the range for biologically activated processes, and indicate volatilization was of

great importance for nitrapyrin loss in these soils.

McCall and Swann (1978) observed the effect of moisture, air-flow, temperature, and soil depth on nitrapyrin volatility. Volatilization was faster in moist soils than dry. This is supported by Briggs (1975), who found that comparing wet soil to dry soil, the initial loss was greater with the dry and that a soil with 5 ml water, applied to the surface, lost somewhat less than a soil with 2.5 ml water because more chemical was moved below the soil surface. Goring (1962b) reports increased water sometimes increases and decreases the effectivity of N-Serve, indicating that for each soil and fertilizer combination there is a particular pattern of water application that will result in optimum control of nitrification.

Little difference in volatilization was observed with different air-flow rates over the soil surface, indicating that once the chemical is incorporated, the rate of volatilization is limited to diffusion of the chemical to the soil surface. Nitrapyrin movement in sandy soils is affected more by volatility than water rate. With increased water rates, higher levels of NH_4^+ move to lower depths than nitrapyrin. This movement of ammonium nitrogen away from the inhibitor zone may account for the reduced effectiveness of nitrapyrin in sandy soils.

Increasing temperatures yield greater losses of nitrapyrin by volatilization. Significant reduction in volatility has been obtained by applying the chemical at deeper soil levels, thus limiting the loss to diffusion of the inhibitor to the soil surface and at the same time the chemical can become more integrated into the soil so equilibrium in the soil matrix is obtained.

Several workers report the effect of increased pH resulting in nitrapyrin becoming less effective (Hendrickson, Walsh, and Keeney, 1978; Goring, 1962a). Conversely, Hendrickson, and Keeney (1979b) using a new bioassay to evaluate the effect of soil properties on nitrapyrin bioactivity, report the nitrifier population to be more susceptible to nitrapyrin as pH increases. When nitrapyrin bioactivity declined, nitrifiers recovered rapidly at high pH. Untreated samples also showed an increased rate of nitrification as pH was increased. "The apparent greater susceptibility of the nitrifiers at high pH would have been difficult to evaluate with other bioassay techniques since observations at later samplings would have shown greater NO_3^- accumulation despite the initial low rate of nitrification. Thus, the rapid recovery of nitrifiers at high pH could have easily masked their greater susceptibility and led to the reported requirement for greater N-Serve concentrations to control nitrification as pH increases (Goring, 1962a)."

Nitrapyrin is marketed in three commercially available forms; N-Serve TG Nitrogen Stabilizer, N-Serve 24 Nitrogen Stabilizer, and N-Serve 24E Nitrogen Stabilizer. N-Serve TG Nitrogen Stabilizer is the technical grade chemical that can be dissolved directly in anhydrous ammonia or methylene chloride, or it can be dissolved in xylene and applied to dry fertilizer for later application. N-Serve 24 Nitrogen Stabilizer is an oil-soluble nonemulsifiable formulation. It may be applied by mixing directly with anhydrous ammonia or it may be mixed with dry fertilizers and applied in a subsurface band. N-Serve 24E Nitrogen Stabilizer is an emulsifiable formulation designed for use in liquid fertilizers such as aqueous ammonia, urea solutions, and

certain mixed salt solutions. This formulation also can be used as an emulsion with water for simultaneous application with any ammonium-type fertilizer. Applications of N-Serve 24E with aqueous fertilizers must use constant agitation to maintain complete emulsion. All formulations of N-Serve should be applied at a recommended minimum soil depth of four inches. The recommended field rate of N-Serve is 0.125-0.25 ppm active ingredient depending on soil conditions and the intended crop.

Nitrapyrin is specifically active against Nitrosomonas, the chemoautotrophic bacterium whose sole energy source rest upon the oxidation of ammonium ion to nitrite in soil. The biological activity of nitrapyrin has been investigated by Campbell and Aleem (1965a), who observed that a concentration as low as 0.20 ppm completely inhibited growth of the organism, and that a concentration of 1.0 ppm cause complete inhibition of ammonia oxidation. This is supported by Goring (1962a,b) who observed that N-Serve concentrations of 0.1-0.2 ppm were effective in slowing ammonium disappearance from fallow fields treated with ammonium fertilizers. Campbell and Aleem (1965a) concluded that the chemoautotrophic metabolism of Nitrosomonas may involve two things: (1) the inhibition of chemosynthetic reactions dependent upon reduction, and (2) a binding or chelating effect upon a metal component of the cytochrome oxidase enzymes involved in ammonia oxidation. The metal involved in substrate oxidation is believed to be copper, as a concentration of 6×10^{-4} M Cu^{++} was found to be effective in a 50 to 70 percent reversal of nitrapyrin inhibition of ammonia oxidation.

Hooper and Terry (1973) classified nitrapyrin's effect on N. europaea as irreversible at 12 ppm (100 percent inhibition) of the chemical. On

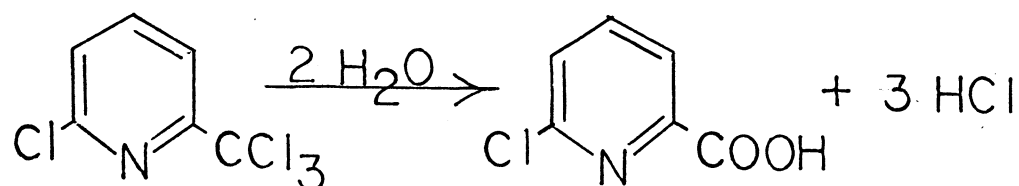
the other hand, Laskowski and Bidlack (1977) report Nitrosomonas recovery from 10 ppm nitrapyrin treatment, and therefore nitrapyrin obviously did not cause complete kill of the organisms. They concluded that Nitrosomonas recovery occurs after dissipation of the chemical below a certain minimum, and that, in the field, broadcast and band applications of nitrapyrin expose only a portion of the soil to the chemical. Thus, there is always untreated soil available to aid in the reestablishment of the nitrifiers. Nitrapyrin soil inhibition, thus acts as a bacteriostat rather than a bacteriocide. Nitrosomonas bacteria would never be eradicated in the field due to the use of nitrapyrin, but once nitrification is inhibited in a certain zone, the resumption of the process in that zone is quite slow and is dependent upon soil pH, organic matter, reinfestation, and temperature (Goring, 1962a; Turner, Warren, and Andriessen, 1962)

Nitrapyrin has not been found to be harmful to other soil organisms when used at recommended rates (Campbell and Aleem, 1965a,b; Goring, 1962a,b). Campbell and Aleem (1965b) reports that concentrations up to 50 ppm of inhibitor exhibit virtually no effect upon nitrite oxidation by Nitrobacter. In the same study, 80-175 ppm N-Serve was slightly inhibitory to nitrite oxidation. The effect on Nitrobacter was very similar to Nitrosomonas inhibition, such as the N-Serve sensitive cytochrome oxidase component of Nitrobacter is probably due to the chelating action of copper. In addition, nitrapyrin may also be inhibitory to other electron transport components, notably the flavins (Campbell and Aleem, 1965b). Rennie (1978) observed a slight sensitivity of Nitrobacter to 10 ppm nitrapyrin in the early log phase of growth,

but after six days a stimulation of multiplication was encountered.

Tu (1973) reports no harmful effect on fungal populations at 20 and 40 ppm nitrapyrin. Shattuck and Alexander (1963) observed no effect on the heterotrophic fungus Aspergillus flavus; the chemoautotrophic bacteria Thiobacillus novellus, Thiobacillus thioparus, and Ferrobacillus sp.; the heterotrophic bacteria Bacillus subtilis, Serratia kilensis, Alcaligenes denitrificans, Aerobacter aerogenes, Achromobacter sp., and Staphylococcus aureus; and no inhibition of the algae Pandorina morum, Chlamydomonas sp., Volvox globator, and Chlorella sp. This would suggest that nitrapyrin action is restricted to only one group of autotrophic microorganisms. Yet Somville (1978) reports an inhibition of bicarbonate incorporation by sulphate-reducing bacteria, however, a concentration of 5 ppm nitrapyrin (well above recommended field rates) was used.

Nitrapyrin is hydrolyzed to 6-chloropicolinic acid with the liberation of three moles of Cl^- :



Hydrolysis is usually regarded as the most important loss mechanism when nitrapyrin has been incorporated in the soil. Hydrolysis is a chemical process rather than biological and is affected more by temperature than pH, thus, nitrapyrin will not hydrolyze over winter (Hendrickson and Keeney, 1979a). Goring (1962a) reports complete control of nitrification for 24 weeks at 50° to 70°F for all concentrations tested. An increase to 90°F gave partial control after eight weeks at

1.0 ppm and after 16 weeks at 5 ppm, but no control after 24 weeks. Touchton, Hoelt, and Welch (1978) report degradation of nitrapyrin not to be affected by nitrapyrin concentration and concentration or form of nitrogen. The workers did find reduced degradation in a silty clay loam soil with high organic matter as compared with a silt loam with low organic matter.

Nitrapyrin and its metabolite, 6-chloropicolinic acid, have been compared as to their relative phytotoxicities. Geronimo et al. (1973) have reported nitrapyrin to be more toxic to Gramineous species (Zea mays L., Sorghum vulgare L., Triticum aestivum L., and Oryza sativa L.), while 6-chloropicolinic acid appears more toxic to dicotyledons (Beta vulgaris L., Lycopersicon esculentum L., Glycine max L., Medicago sativa L., and Gossypium hirsutum L.). Dicots also appear to be somewhat sensitive to nitrapyrin.

When comparing the two compounds with reference to exposure sites of wheat and cotton seedlings, Geronimo, Smith, and Stockdale (1973) found that the site of exposure of the germinating seedlings to the chemical influences the degree of phytotoxicity obtained, although the inherent activity of each compound against each species appears to be a more important factor with regard to phytotoxicity. Nitrapyrin reduced top growth of both cotton (minimum concentration of 20 ppm) and wheat (minimum concentration of 10 ppm) when exposure occurred through both roots and shoot, while 6-chloropicolinic acid reduced top growth of both species when exposure occurred only through the root.

Comparison studies of the two nitrification inhibitors, potassium azide and nitrapyrin, most often show the latter to be superior.

Parr, Carroll, and Smith (1971) report nitrapyrin to be superior to KN_3 in incubation studies when both are formulated with anhydrous ammonia at 10 ppm inhibitor. Nitrapyrin was thought to be more effective than KN_3 because of its greater residual activity, since KN_3 approached nitrapyrin's level of effectiveness only during the first two weeks of incubation. Kapusta and Varsa (1972) report nitrapyrin was more effective, especially at 2 pt./acre, than KN_3 in promoting increased corn yield with 100 lb. N/acre anhydrous ammonia.

Studying transformation of urea N in soil, Bundy and Bremner (1974) and Bremner and Bundy (1976) showed that, unlike N-Serve, KN_3 retards urea hydrolysis in soils, but does not prevent the accumulation of nitrites in soils that accumulate nitrite when treated with urea alone. It was concluded that KN_3 , when applied with urea to soils that normally accumulate nitrite, is decomposed by reaction with the nitrite.

The two inhibitors were tested by Henninger and Bollag (1976) to determine their effect on denitrification by a Pseudomonas sp. in pure culture and in soil. In culture, N-Serve exerted a strong inhibitory effect on nitrate reduction at 50 ppm; below 30 ppm N-Serve did not affect denitrification. KN_3 showed no inhibition of denitrification in culture. In soil, nitrapyrin had no effect on denitrification. This difference indicates that a chemical may have no noticeable effect on the microbial population as a whole, but it can affect the activity of individual microorganisms. KN_3 , in soil, strongly inhibited the transformation of N_2O to N_2 .

Incubation studies by Goring (1962a) report N-Serve controlled nitrification for four weeks at 0.2 ppm, 8 weeks at 0.5 ppm, and 12 weeks

at 1.0 ppm. Control samples without N-Serve were completely nitrified at four weeks. He concluded that the minimum concentration of N-Serve for a 6 week inhibition varies from 0.2 to 2.0 ppm, the concentration being dependent on soil properties. Boswell and Anderson (1974) support these findings with their incubation studies with soil contained in field-buried polyethylene bags. Nitrification was inhibited for a four month period using ammonium nitrate (70 ppm-N) in conjunction with nitrapyrin (1.0 ppm).

Page (1975) investigated the persistence of anhydrous and aqueous ammonia in conjunction with N-Serve on a sandy loam soil. The rate of decay of both aqueous and anhydrous ammonia was approximately 1 percent per day at 0°C and had a Q_{10} of 2.1. In application with N-Serve (1.5 percent) on anhydrous ammonia, the rate of decay was approximately halved.

N-Serve has been used with various nitrogen fertilizers in an attempt to increase yields from crop plants. Both success and failure have been reported with the results largely dependent on the type of fertilizer, concentration of N-Serve, soil texture, soil moisture, soil organic matter, and soil pH.

Soil conditions which normally tend to contribute to a yield increase with N-Serve are: wet soil due to a high water table or slow permeability in wet weather; very porous soil where leaching may be excessive, especially if the soil has a high moisture content at the beginning of a rainfall; and soil with a high amount of easily oxidizable organic matter, especially if the soil is wet or the ratio of carbon to nitrogen in the organic matter is high. The organic matter

may be a mulch, as in no-tillage, or crop residue, or a cover crop which has been plowed under. All of these factors tend to increase leaching, denitrification, or immobilization of nitrogen (Frye et al., 1980).

A response to N-Serve may not be obtained if the weather is dry in the spring and early summer, because nitrogen losses would be less. Also, if an adequate amount of nitrogen is supplied to a crop by fertilizer and mineralization of nitrogen from organic matter throughout the growing season, a response or yield increase may not be expected.

McKell and Whalley (1964) reports reducing top and root growth of Medicago sativa L. (inoculated with Rhizobium meliloti) when grown with 1.0, 10, and 20 ppm N-Serve, both with and without nitrogen fertilization. The 20 ppm concentration had a marked effect on root tip and nodule formation, with tumor-like swellings forming just behind the root tips. Only one large nodule was found showing hemoglobin development, while all others were small and white. It was suggested that growth reduction resulted from the interference of normal root cell division and tissue differentiation, which concomitantly reduced water and nutrient adsorption by the deformation of root tips.

Phytotoxicity of N-Serve has also been reported to effect other crops. Mills et al. (1973) report 50 ppm N-Serve to be toxic to bean, corn, cucumber, pea, and pumpkin, while no injury to tomato has occurred at 100 ppm inhibitor. Osborne (1977) found nitrapyrin to be phytotoxic to ryegrass (Lolium rigidum L.) and subterranean clover (Trifolium subterraneum L.) at as low as 5 ppm inhibitor. Increased phytotoxicity was observed at 10 and 50 ppm inhibitor.

The chemical itself may have been toxic or, considering the work of Gasser, Greenland, and Rawson (1967), the phytotoxicity may have been caused by changes that the inhibitor induced in the proportions of ammonium and nitrate available to the plant. Gasser (1965), using ammonium sulphate (50 ppm) and nitrapyrin at lower levels (0.5 and 1.0 ppm), reports increases in yield of dry matter with ryegrass grown on both sandy and clay loam soil.

Increased corn (Zea mays L.) yield has been reported by numerous researchers. Huffman (1979) found N-Serve increased yield by an average of 12 bu./acre, with a low of 5.8 bu./acre and a high of 25.0 bu./acre. Early seasonal and mid-seasonal varieties averaged 13.4 more bushels per acre with N-Serve, while full season varieties averaged 3.6 more bushels per acre. In areas where summer rainfall is erratic and minimal, the response of early-mid-season maturing corn to N-Serve could be very significant. Irrigated corn tended to have a yield increase near that of the average and it was observed that all treatments of corn with N-Serve tended to silk earlier and more uniformly, and also show a degree of drought tolerance as compared to the untreated corn. Warren et al. (1975) support the results of Huffman (1979). They report increases of grain yield and grain protein from N-Serve (0.5 ppm) with Fall applied anhydrous ammonia. Grain yield was increased an average of 68 percent and as much as 207 percent, while grain protein increased 7-38 percent. Conversely, White, Hoeft, and Touchton (1978) report nitrapyrin did not increase yield or stalk diameter with N-Serve at rates of 0.56 and 1.0 kg/ha. Boswell (1977) found nitrapyrin not to have any influence on yield, number of ears, average ear weight,

and percent N, P, K, Ca, Mg, Mn, Fe, and Zn when fertilized with anhydrous ammonia (90 and 180 kg/ha) and N-Serve (2,338 ml/ha).

Cotton yield has been increased by N-Serve in most studies. Swezey and Turner (1962) report that a single application of 100 lb. urea N/acre with 1.0 ppm N-Serve, gave a higher yield than double the rate of untreated fertilizer applied as two side dressings. Increased yield with one application was also observed by Turner and Nilson (1964). Their increase resulted in 0.06-0.07 more bales/acre and a result of a gross increase of 10 to 12 dollars per acre. Huffman (1979) observed that N-Serve, applied with preplant nitrogen, appears to have a positive effect on stimulating seedling vigor, and under the adverse cool wet soil conditions involved, developed a better root system.

Nitrate accumulation has been lowered in lettuce and spinach, which normally accumulate nitrates in their leaves, by the use of N-Serve (Moore, 1973). Accumulation of nitrate in Raphanus sativus L. has been eliminated with 50 ppm nitrapyrin. The increased ammonium made available to radish increases shoot growth and retards (20-25 percent) root growth relative to nitrate nitrogen. This is probably due to ammonium toxicity in the roots. The roots are most likely able to detoxify the ammonium by incorporating it into amino acids and amides, thus using up the carbohydrate reserves of the roots. Likewise, in the shoot, the assimilation of nitrate may utilize the carbohydrate reserves of the shoot. Nitrate accumulation in the plant was found to be minimal even with generous ammonium application, and accumulation is primarily in the shoot under this system of fertilization (Mills et al., 1976).

Increased rice yield has been obtained by Wells (1977). Urea fertilization, in conjunction with nitrapyrin (0.5 and 1.0 ppm), increased grain yield 500 to 700 lb./acre. Protein content was also increased. This is supported by Sahrawat and Mukerjee (1976) who found a significant increase in grain protein with nitrapyrin (0.75 ppm) plus urea or ammonium sulphate (135 ppm-N). Narain and Datta (1974), in pot experiments report 150 kg/ha ammonium sulphate or urea was superior to ammonium nitrate for rice, but the addition of 5 ppm N-Serve had no significant effect on rice yield. The researchers concluded that continuous water-logging impaired nitrification and masked the effect of N-Serve, and that no leaching losses of nitrate occurred.

Sugarcane (Saccharum officinarum) has been found to grow better on ammonium nitrogen than nitrate nitrogen. In field experiments by Prasad (1976), 51.5 ppm N of ammonium sulphate in conjunction with 1.3 ppm N-Serve gave yields almost equal to fertilization with 103 ppm N ammonium sulphate without N-Serve.

Huber, Murray, and Crane (1969) report N-Serve (0.5 and 0.6 ppm) in conjunction with ammonium sulphate, increased yield of wheat (Triticum aestivum) 37 to 42 percent, and observed no increase in yield with calcium nitrate. Conversely, Osborne (1977) found 10 ppm N-Serve to inhibit maturity of wheat. Narain and Datta (1974); and Boswell, Nelson, and Bitzer (1976) report neither increased yield nor increased nitrogen levels in tissue or grain of wheat using nitrapyrin (10 and 1.0 ppm respectively). Spratt (1973) does report an increase in phosphate uptake by wheat using nitrapyrin. Theoretically, the efficiency of phosphate fertilizers should be increased if the persistence of ammonium

can be extended.

The use of nitrapyrin as an inhibitor of various crop diseases, primarily potato scab and corn stalk rot, has been investigated. Reduced incidence of potato scab (Streptomyces scabies) has been reported by Potter, Norris, and Lyons (1971). U.S. Number 1 potatoes (Solanum tuberosum L.) had less disease incidence and yield was increased significantly with 2.5 ppm N-Serve and a 55-60-180 (NPK) fertilizer. The researchers concluded that high ammonium and low nitrate levels were important in disease reduction. Other researchers (Davis et al., 1974; Davis, McDole, and Callihan, 1976) report N-Serve increasing disease severity and reducing the levels of Mn, Cu, Mg, Zn, and K and increasing boron level in tuber peelings. It is suggested that the ammonium nitrogen form may influence scab by an effect on calcium and or phosphate. Calcium was shown to have a positive correlation with scab, whereas phosphate-P showed a negative correlation. Ammonium sulphate significantly lowered the calcium:phosphate-P ratio as compared with the use of calcium nitrate. Similar results with N-Serve, in the presence of calcium nitrate and sulfur, reduced calcium and calcium:phosphate-P ratios in tuber peelings. This indicates that the effect of N-Serve is not limited to the ammonium form-of-nitrogen and suggest a relationship between calcium level and sulfur. The presence of sulfur was required to reduce calcium and calcium:phosphate-P ratios and thus suggest that the effects may be partially due to soil pH. MC Gregor and Wilson (1966) associated increased manganese with decreased scab and have suggested that scab reduction may be related to manganese absorption. Hendrickson et al. (1978) evaluated development of potato

tubers in the presence of nitrapyrin and found reduced tuber yield and a reduced proportion of marketable tubers. It was concluded that the high ammonium levels, resulting from N-Serve, interfered with plant metabolism so that yield and normal development of the tubers was impaired.

Nitrapyrin is reported to reduce stalk rot incidence in corn caused by the fungi Diplodia zeae (Schw) Lev. and Gibberella zeae (Schw) Petch. Warren et al. (1975) and White, Hoeft, and Toudhton (1978) report a 60-96 percent reduction in stalk rot using 0.55 and 1.0 ppm nitrapyrin. Many workers have demonstrated that stalk rot development is correlated with cell senescence. Any factor delaying senescence in corn stalks should reduce the severity of rot. Therefore, reduced stalk rot with increased nitrogen is probably due to the plants having an adequate supply of nitrogen throughout the growing season and , therefore, are more resistant than plants which have an adequate supply early in the season and a deficiency late in the season. It was noticed by the researchers that the effect of nitrapyrin was more evident with stalk rot resulting from natural infection of the pathogen. This is possibly due to the inoculating process bypassing the resistance or susceptibility of the root system and not exactly duplicating natural infection.

MATERIALS AND METHODS

Two soil types collected from the top twenty centimeters of the A-horizon were used in this study (table 6). Each soil was air-dried and crushed to pass a U.S. # 10 standard sieve with a pore size of 2 mm. Texture analysis was done by the hydrometer method (Bouyoucos, 1962); percent organic matter and pH (1:2 soil-water ratio) were determined by the procedures described by Page (1965); ammonia nitrogen was extracted by the method of Page (1965) using a 0.05 N HCl and 0.025 N H₂SO₄ extraction solution, and the determination of ammonia nitrogen was done using an Orion (model 407A) specific ion meter (Orion, 1978a); and nitrate extraction and determination were accomplished using the same ion meter following the Orion method (Orion, 1978b).

Urea and ureaformaldehyde fertilizers were applied at concentrations of 25, 38, and 50 ppm-N. N-Serve 24E (NI) concentrations were 0.25, 0.50, and 1.0 ppm active ingredient. For each soil, the experimental design allowed six trials to correspond with incubation periods of 0, 2, 3, 4, 5, and 6 weeks. Soil treatments for each trial are shown in Table 7. All treatments were replicated three times for each trial. The soil (3600 g for all six trials) for each treatment was placed in a shallow tray. The treatment suspension (10 ml liquid per 100 g soil) was applied directly to the soil surface and incorporated using a small trowel. After air-drying over night, each soil treatment was remixed and placed in styrofoam cups. All the cups were brought to field capac-

ity (by weight) with distilled water. The cups were then placed in a room illuminated with cool white, power groove fluorescent bulbs and 100 watt incandescent bulbs. The light intensity at table top was 400 ft-c with a 16-hour photoperiod. Room temperatures were maintained at 80-85°F during the light period, and 70-75°F during the dark period. Cups were arranged by weeks and rotated randomly every two days following watering to field capacity.

At the end of a trial's incubation period, the soil from each cup was mixed and approximately thirty-five grams (wet weight) of soil was removed from each cup and placed in a plastic petri-dish. The samples were dried overnight at 45°C and then sampled for ammonia and nitrate nitrogen. The remaining soil was placed back in each cup, and twenty seeds of perennial ryegrass (Lolium perenne L.) were planted in the soil at a depth of one centimeter. The ryegrass plants were harvested seventeen days after planting by cutting the plants off at the soil surface. Fresh weights (two replicates per cup) were recorded, and the excised plants were placed in freezer bags and refrigerated at 2°C until analysis for chlorophyll (mg/g fresh weight). Total chlorophyll was determined by the method of Arnon (1949).

RESULTS AND DISCUSSION

NITRIFICATION INHIBITION IN INCUBATION EXPERIMENTS

The apparent nitrification rates in these experiments have been estimated by the rate of $\text{NH}_3\text{-N}$ disappearance, NO_3^- -N accumulation, and the total recovery of NH_3 and NO_3^- nitrogen.

Sandy Loam Soil

Hydrolysis of urea, at all three concentrations, to ammonium was nearly complete at fourteen days (figure 1). Nitrate nitrogen decreased during the first fourteen days at urea concentrations of 25 and 50 ppm-N. Nitrate in the 38 ppm-N treatment increases slowly during the first fourteen days, but the increase does not correspond to the decrease in $\text{NH}_3\text{-N}$ (figures 1 and 2). The low recovery of nitrate is most likely due to the immobilization of nitrate into organic matter by the heterotrophic flora. The recovery of applied nitrogen at fourteen days is neither accounted for as ammonia nor nitrate nitrogen (table 8). Nitrate nitrogen at this point is probably immobilized in soil organic matter. The $\text{NH}_3\text{-N}$ has either been incorporated into the soil organic matter, lost by volatilization, or converted to nitrite. Soil organic matter incorporation of $\text{NH}_3\text{-N}$ is probably minimal (Brady, 1974). Since the soil was maintained at field capacity, loss by volatilization is also probably minimal. The greatest amount of non-recovered nitrogen is probably in the form of nitrite. Fertilization with ammonia nitrogen increases the pH and may cause a delay of the conversion of nitrite to

nitrate until after the ammonium ion concentration is reduced to a relatively low level (Brady, 1974). The maintenance of soil water at field capacity may have aided in nitrite accumulation, since nitrite accumulates in anaerobic soils. At all concentrations, nitrate levels increased steadily from fourteen to thirty-five days. During this time, the accumulated nitrite is probably being converted to nitrate. Applied nitrogen not recovered in the final twenty-eight days of incubation could possibly be attributed to loss by denitrification.

Addition of N-Serve at the 0.50 and 1.0 ppm levels extended the persistence of $\text{NH}_3\text{-N}$ in all urea treatments (figures 3, 4, 5, 6, 7, and 8). The 0.25 ppm N-Serve concentration had no effect on maintaining $\text{NH}_3\text{-N}$ persistence. The 0.50 ppm level of the inhibitor extended the persistence of $\text{NH}_3\text{-N}$ to twenty-one days for all urea concentrations. The 1.0 ppm N-Serve treatment maintained the $\text{NH}_3\text{-N}$ level for approximately twenty-one days and extended $\text{NH}_3\text{-N}$ persistence twenty-eight days. As in the soil treated with urea alone, nitrate accumulation did not begin until fourteen days and then increased as the remaining ammonium was nitrified. Nitrogen not recovered (table 8) at the end of the forty-two day incubation time is associated with denitrification.

At 1.0 ppm N-Serve, nitrification was completely inhibited for fourteen days, and partial control was maintained for at least twenty-one days. This is in accordance with the findings of Hendrickson et al. (1978), who observed complete inhibition of nitrification for fifteen days, and partial control for at least forty-nine days at 1.0 ppm N-Serve. The difference in persistence times is probably due to variation in technique leading to greater hydrolysis of the N-Serve in this study.

The three concentrations of ureaformaldehyde exhibited nearly the same initial amount of fast release (fraction I) nitrogen (figures 9 and 10). This ammonium was almost completely nitrified by fourteen days. Nitrate accumulation was minimal until fourteen days, thus indicating nitrite accumulation in the soil. Nitrogen from the slow release fractions of the ureaformaldehyde is evident from fourteen to thirty-five days, at which time any $\text{NH}_3\text{-N}$ released is immediately nitrified to $\text{NO}_3^-\text{-N}$. At thirty-five days, the recovery (table 8) of nitrogen is less than that applied. This may be due to denitrification or nitrogen which has yet to be released from fractions II and III of the ureaformaldehyde.

Clay Loam Soil

Persistence of $\text{NH}_3\text{-N}$ in the 25 ppm-N urea treatment was at least twenty-eight days (figure 11). Persistence of $\text{NH}_3\text{-N}$ in the 38 and 50 ppm-N treatments lasted throughout the duration of the forty-two day incubation period. Nitrate levels in the urea treatments increased from day zero and gave no evidence of nitrite accumulation occurring in this soil (figure 12). Recovery of applied nitrogen is shown in table 9. Losses of nitrogen are attributed to denitrification.

All combinations of urea and N-Serve, except the 38 ppm-N urea and 0.25 ppm N-Serve treatment, maintained $\text{NH}_3\text{-N}$ levels for essentially twenty-eight days, and partial control was obtained for approximately thirty-five days. The 38 ppm-N urea and 0.25 ppm N-Serve treatment maintained partial control for at least 21 days (figures 13, 14, 15, 16, and 17). Nitrogen recovery for the N-Serve treatments is shown in table 9.

The three concentrations of ureaformaldehyde exhibited the same response as in the sandy loam soil (figures 19 and 20). Initial release $\text{NH}_3\text{-N}$ was nearly the same for all concentrations of ureaformaldehyde. Nitrate accumulation began at fourteen days. Ammonium released after fourteen days is immediately converted to nitrate by nitrification.

Comparison of the Two Soils

Persistence of applied $\text{NH}_3\text{-N}$, with or without a nitrification inhibitor, is dependent upon soil characteristics. Rapid nitrification of $\text{NH}_3\text{-N}$ in the sandy loam (figure 1) is to be expected as it is well aerated because of its texture. The clay loam soil; on the other hand, has a high clay content which binds the ammonium cations in a nonexchangeable form (Brady, 1974). This ammonium is slowly released and may account for the slower nitrification rates of the clay loam soil (figure 11).

The increased maintenance of $\text{NH}_3\text{-N}$ levels with N-Serve treatment in the clay loam is superior to the sandy loam soil. The low organic matter content of the sandy loam soil likely resulted in limited adsorption of the N-Serve, thus maintaining relatively high concentrations of N-Serve in both the solution and the vapor phase of the soil. The high levels of N-Serve in solution and in the vapor phase would promote the inactivation of the N-Serve by increasing hydrolysis and volatilization. The high organic matter found in the clay loam soil probably increased the adsorption of the N-Serve and decreased its susceptibility to volatilization and hydrolyzation. Goring (1962a) reports that soil with high organic matter requires more of the chemical to inhibit nitrification. These data suggest that the high organic matter was important

in retarding the loss of the N-Serve from volatilization and hydrolyzation.

CHLOROPHYLL ANALYSIS

Sandy Loam Soil

Analysis of total chlorophyll for ryegrass plants grown in the sandy loam soil is shown in table 10. Plants in growth period one contained the highest level of chlorophyll when compared with the other three periods. Plants in the first growth period were germinated and established during the period of time when ammonium nitrogen was at its highest concentration (figures 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10) in all treatments. This corresponds with reports that members of the Gramineae, when young, respond better to ammonium than nitrate source of nitrogen (Bidwell, 1979).

Chlorophyll content increased with increasing concentrations of urea throughout the four growth periods. Plants in growth period three contained less chlorophyll than the other periods. A possible explanation for this occurrence is the accumulation of nitrite up to the fourteenth day. The level of nitrite may be high enough to adversely effect the germinating ryegrass plants and, therefore may have reached toxic levels.

All combinations of N-Serve and urea, except the lowest N-Serve and urea combination, increased the chlorophyll content of the ryegrass more than the urea alone during the first two growth periods. In the second growth period, only the N-Serve treatments of 1.0 ppm significantly raised the chlorophyll content more than that of the urea.

SUMMARY

Control of nitrification was obtained in both soils by the use of the nitrification inhibitor, N-Serve. N-Serve concentrations of 0.50 and 1.0 ppm were effective in controlling nitrification of applied urea nitrogen in both of the soils tested. The 0.25 ppm N-Serve concentration was effective in only the clay loam soil. Maintenance of applied nitrogen in the $\text{NH}_3\text{-N}$ form by N-Serve was greatest in the clay loam soil. Evidence has been presented by many researchers that high organic matter adsorbs and thus inactivates the N-Serve (Goring, 1962a; Lewis and Stefanson, 1975; Hendrickson et al., 1978). Laboratory experiments by Goring (1962a) indicate that N-Serve is most effective on coarse-textured soils with low organic matter. The results of this study indicate better control in the finer-textured soil with a high organic matter content. The increased $\text{NH}_3\text{-N}$ persistence in the high organic clay loam soil, as compared to that of the low organic sandy loam soil, was probably due to organic matter adsorption of the N-Serve. Adsorption of the inhibitor possibly maintained it in the soil for a longer period of time and did not allow volatilization of the chemical as readily as in the sandy loam soil. The greater persistence of $\text{NH}_3\text{-N}$ in the clay loam soil was also aided by the high clay content of that soil. The clay's holding capacity of ammonium ions decreases the availability of the ion to nitrification. Both clay content and decreased volatilization of N-Serve are responsible for the increased persistence of $\text{NH}_3\text{-N}$ in

the clay loam soil.

Chlorophyll content of ryegrass plants increased in all treatments for both soils during the periods of time in which the ammonium concentrations were highest. In the sandy loam soil, the urea treatments without N-Serve and the ureaformaldehyde treatments increased chlorophyll content of the ryegrass seedlings at approximately the same rate throughout all the growth periods. The addition of N-Serve to urea increased the chlorophyll content of the plants more than either the urea without inhibitor or the ureaformaldehyde treatments in the first two growth periods.

In the clay loam soil, the ureaformaldehyde treatments did not significantly increase chlorophyll content at any concentration throughout the four growth periods. Urea treatments, with or without N-Serve, increased chlorophyll content significantly in the first two growth periods. The greatest increase in chlorophyll was at the 1.0 ppm N-Serve concentration.

The recovery of nitrogen from the ureaformaldehyde treatments in this study is not completely accurate since organic nitrogen was not measured. However, the nitrogen from the ureaformaldehyde which was recovered was mostly in the NO_3^- -N form. According to the data presented in this study, NO_3^- -N does not enhance the synthesis of chlorophyll in establishment of ryegrass seedlings, whereas, ammonium nitrogen seemed to be positively correlated to the synthesis of chlorophyll in the ryegrass seedlings.

APPENDIX

Table 1. Nitrogen fractions of a commercial ureaformaldehyde.

Fraction	% of Total	<u>No. ureas</u> molecule	Period of Release
I Cold Water Soluble	32.6	2-3	A Few Weeks
II Soluble Hot; Insoluble Cold	32.9	4-5	Several Months
III Hot Water Insoluble	34.5	7-8	1-2 Years

Table 2. Recovery of fertilizer nitrogen from coastal bermudagrass over an eight month period in greenhouse experiments.

<u>Source</u>	<u>% Recovery of N¹⁵ (N[*])</u>			
	<u>Tops</u>	<u>Roots</u>	<u>Soil</u>	<u>Total Recovery</u>
N [*] H ₄ NO ₃	69.8	2.7	16.7	89.2
NH ₄ N [*] O ₃	77.3	3.0	9.1	89.4
UF	55.2	3.0	32.2	90.4

Table 3. Recovery of ureaform nitrogen by Alta Fescue (from Hays).

<u>Material</u>	<u>% Applied N Recovered in 26 Weeks</u>				
	<u>Clippings</u>	<u>Crown</u>	<u>Root</u>	<u>Soil</u>	<u>Total</u>
NH_4NO_3	43.4	33.7	4.6	11.0	92.7
	(6 weeks)	(13 weeks)			
Nitroform	28.6	10.2	9.0	48.2	96.6
Nitroform fractions					
I	57.8	18.4	7.8	18.6	89.3
II	33.3	20.8	6.8	45.5	93.4
III	2.1	7.8	8.1	71.3	87.7
II + III	20.2	18.0	9.0	56.2	92.0

Table 4. Solubility of nitrapyrin.

Solvent	Temperature°C	Grams/100 g Solvent
Acetone	20	198
Andrydrous ammonia	33	0.33
Anhydrous ammonia	0	6-9
Anhydrous ammonia	10	18-25
Anhydrous ammonia	22	54-67
Ethanol	22	30
Methylene chloride	20	185
Xylene	26	104
Water	22	0.004

Table 5a. Vapor pressure of nitrapyrin dissolved in various solvents.

Solvent	Temperature°C	V.P. (mm Hg)
Xylene	4	2.8×10^{-3}
Anhydrous ammonia	10	1.07×10^{-2}
Water	42	1.0

Table 5b. Comparative vapor pressure of nitrapyrin and the herbicide trifluoralin.

Chemical	Temperature°C	V.P. (mm Hg)
Nitrapyrin	20	2.8×10^{-3}
Trifluoralin	29.5	1.99×10^{-4}

Table 6. Properties of the two soils used in this study.

Soil	Mechanical Analysis			% Organic matter	pH
	% sand	% clay	% silt		
I Sandy loam	56	14	30	1.61	7.01
II Clay loam	27	32	41	5.55	5.58

Table 7. Soil treatments for each trial.

Treatment	N-Serve (NI) (ppm)	Urea (ppm-N)	Ureaformaldehyde (ppm-N)
Control	0	0	0
Urea	0	25	0
Urea	0	38	0
Urea	0	50	0
Urea-NI	0.25	25	0
Urea-NI	0.50	25	0
Urea-NI	1.0	25	0
Urea-NI	0.25	38	0
Urea-NI	0.50	38	0
Urea-NI	1.0	38	0
Urea-NI	0.25	50	0
Urea-NI	0.50	50	0
Urea-NI	1.0	50	0
Ureaformaldehyde	0	0	25
Ureaformaldehyde	0	0	38
Ureaformaldehyde	0	0	50

Table 8. Recovery of $\text{NH}_3\text{-N}$ and $\text{NO}_3^-\text{-N}$ from all treatments of the sandy loam soil. The control has been subtracted from the treatment data.

Days		0	14	21	28	35	42
Treatment							
Control	$\text{NH}_3\text{-N}$	5.99	2.46	5.33	5.06	3.05	4.22
	$\text{NO}_3^-\text{-N}$	3.15	1.56	13.70	17.73	19.29	17.75
	Total	9.14	4.02	19.03	22.79	22.34	21.97
Urea 25 ppm-N	$\text{NH}_3\text{-N}$	19.19	0.60	0.51	0	0.84	0.05
	$\text{NO}_3^-\text{-N}$	2.49	1.68	3.49	5.28	19.47	8.69
	Total	21.68	2.28	4.00	5.28	10.31	8.74
Urea 38 ppm-N	$\text{NH}_3\text{-N}$	31.21	1.60	1.28	0	1.63	0.33
	$\text{NO}_3^-\text{-N}$	0	0.47	7.86	11.42	13.71	13.20
	Total	31.21	2.07	9.14	11.42	15.34	13.53
Urea 50 ppm-N	$\text{NH}_3\text{-N}$	38.18	1.15	1.65	0	1.75	0.06
	$\text{NO}_3^-\text{-N}$	4.47	1.83	12.56	23.55	24.55	20.19
	Total	42.65	2.98	14.21	23.55	26.30	20.25

Table 8. (cont.)

Days		0	14	21	28	35	42
Treatment							
Urea 25 ppm-N	NH ₃ -N	23.91	0.71	0.04	0	1.27	0
NI 0.25 ppm	NO ₃ -N	3.92	1.52	8.75	18.43	16.10	20.46
	Total	27.83	2.23	8.79	18.43	17.37	20.46
Urea 25 ppm-N	NH ₃ -N	24.63	10.82	1.42	0	1.47	0
NI 0.50 ppm	NO ₃ -N	0.59	0.98	12.86	11.97	16.94	24.04
	Total	25.22	11.80	14.28	11.97	18.41	24.04
Urea 25 ppm-N	NH ₃ -N	24.90	23.16	12.39	0	1.58	0
NI 1.0 ppm	NO ₃ -N	0.15	0.03	7.47	16.68	16.69	23.88
	Total	25.05	23.19	19.86	16.68	18.27	23.88
Urea 38 ppm-N	NH ₃ -N	27.48	1.15	0.44	0	1.73	0
NI 0.25 ppm	NO ₃ -N	0	2.81	23.53	16.58	25.11	29.03
	Total	27.48	3.96	23.97	16.58	26.84	29.03

Table 8. (cont.)

Days		0	14	21	28	35	42
Treatment							
Urea 38 ppm-N	NH ₃ -N	26.87	18.70	1.02	0	1.91	0
NI 0.50 ppm	NO ₃ ⁻ -N	0	0.48	20.79	15.12	25.61	22.13
	Total	26.87	20.18	21.81	15.12	27.52	22.13
Urea 38 ppm-N	NH ₃ -N	27.92	28.98	24.85	0.61	2.29	0.07
NI 1.0 ppm	NO ₃ ⁻ -N	0	0	11.94	15.24	23.45	17.57
	Total	27.92	28.98	36.79	15.85	25.74	17.64
Urea 50 ppm-N	NH ₃ -N	32.91	2.66	1.67	0.31	2.87	0.78
NI 0.25 ppm	NO ₃ ⁻ -N	0	2.51	20.70	17.30	35.85	26.43
	Total	32.91	5.27	22.37	17.61	38.72	27.21
Urea 50 ppm-N	NH ₃ -N	36.91	17.46	1.94	0.50	2.70	0.41
NI 0.50 ppm	NO ₃ ⁻ -N	0	0.99	24.04	19.77	34.80	29.26
	Total	36.91	18.45	25.98	20.27	37.50	29.67

Table 8. (cont.)

Days		0	14	21	28	35	42
Treatments							
Urea 50 ppm-N NI 1.0 ppm	NH ₃ -N	34.83	35.18	24.63	1.38	2.88	0.34
	NO ₃ ⁻ -N	0	0	15.39	16.63	39.62	29.11
	Total	34.83	35.18	40.02	28.01	42.50	29.45
UF 25 ppm-N	NH ₃ -N	15.35	0.69	0	0	0.32	0
	NO ₃ ⁻ -N	0	0.95	23.86	18.61	25.97	19.54
	Total	15.35	1.64	23.86	18.61	26.29	19.54
UF 38 ppm-N	NH ₃ -N	18.26	0.77	0	0	0.46	0
	NO ₃ ⁻ -N	0	1.04	23.59	14.74	29.83	25.88
	Total	18.26	1.81	23.59	14.74	30.29	25.88
UF 50 ppm-N	NH ₃ -N	16.83	0.48	0	0	0.38	0
	NO ₃ ⁻ -N	0	1.53	26.28	17.62	40.37	28.58
	Total	16.83	2.01	26.28	17.62	40.75	28.58

Table 9. Recovery of $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ from all treatments of the clay loam soil. The control has been subtracted from the treatment data.

Days		0	14	21	28	35	42
Treatment							
Control	$\text{NH}_3\text{-N}$	13.28	35.06	34.57	13.91	11.80	11.93
	$\text{NO}_3\text{-N}$	5.95	11.00	27.89	21.55	31.19	29.86
	Total	19.23	36.06	62.46	35.46	42.99	41.79
Urea 25 ppm-N	$\text{NH}_3\text{-N}$	20.19	9.71	4.50	5.95	0	1.84
	$\text{NO}_3\text{-N}$	0	1.79	0	3.37	1.55	5.73
	Total	20.19	11.50	4.50	9.32	1.55	7.57
Urea 38 ppm-N	$\text{NH}_3\text{-N}$	21.72	17.72	15.70	13.19	7.51	5.70
	$\text{NO}_3\text{-N}$	0	4.58	3.67	8.03	12.69	13.03
	Total	21.72	22.30	19.37	21.22	20.20	18.73
Urea 50 ppm-N	$\text{NH}_3\text{-N}$	34.78	26.28	23.65	18.79	8.94	9.20
	$\text{NO}_3\text{-N}$	0	7.40	7.25	12.56	16.95	14.16
	Total	34.78	33.68	30.90	31.35	25.89	23.36

Table 9. (cont.)

Days		0	14	21	28	35	42
Treatment							
Urea 25 ppm-N NI 0.25 ppm	NH ₃ -N	17.39	17.56	20.90	16.77	8.75	4.61
	NO ₃ ⁻ -N	0.51	0	0	9.74	10.53	9.76
	Total	17.90	17.56	20.90	26.51	19.28	14.37
Urea 25 ppm-N NI 0.50 ppm	NH ₃ -N	17.17	19.69	21.01	21.37	14.10	5.14
	NO ₃ ⁻ -N	1.07	0	0	12.20	6.18	16.24
	Total	18.24	19.69	21.01	33.57	20.28	21.38
Urea 25 ppm-N NI 1.0 ppm	NH ₃ -N	31.92	19.97	18.82	21.37	9.49	2.97
	NO ₃ ⁻ -N	0	0	0	13.07	10.98	13.67
	Total	31.92	19.97	18.82	34.44	20.47	16.64
Urea 38 ppm-N NI 0.25 ppm	NH ₃ -N	39.83	16.85	20.52	7.96	0.83	0
	NO ₃ ⁻ -N	0	0	5.33	20.50	19.14	19.41
	Total	39.83	16.85	25.85	28.46	19.97	19.41

Table 9. (cont.)

Days		0	14	21	28	35	42
Treatment							
Urea	NH ₃ -N	37.74	24.75	29.41	25.04	14.48	3.43
38 ppm-N							
NI 0.50 ppm	NO ₃ ⁻ -N	0	0	0.88	7.84	16.47	26.49
	Total	37.74	24.75	32.29	32.88	30.95	29.92
Urea	NH ₃ -N	32.31	28.15	34.24	33.93	21.34	4.70
38 ppm-N							
NI 1.0 ppm	NO ₃ ⁻ -N	0	0	0	7.87	16.42	16.68
	Total	32.31	28.15	34.24	41.80	37.76	21.38
Urea	NH ₃ -N	34.06	26.89	32.65	23.02	11.90	6.84
50 ppm-N							
NI 0.25 ppm	NO ₃ ⁻ -N	0	0	3.24	17.61	20.79	27.09
	Total	34.06	26.89	35.89	40.63	32.69	33.93
Urea	NH ₃ -N	38.40	32.86	39.18	33.72	17.28	3.87
50 ppm-N							
NI 0.50	NO ₃ ⁻ -N	0	0	5.30	12.06	27.72	33.27
	Total	38.40	32.86	44.48	45.78	45.00	37.14

Table 9. (cont.)

Days		0	14	21	28	35	42
Treatment							
Urea 50 ppm-N NI 1.0 ppm	NH ₃ -N	37.97	29.52	36.54	32.18	8.01	0.15
	NO ₃ ⁻ -N	0	0	8.51	16.46	32.09	35.41
	Total	37.97	29.52	45.05	48.64	40.10	35.56
UF 25 ppm-N	NH ₃ -N	8.55	0	0	0	0	0
	NO ₃ ⁻ -N	0	0	10.15	22.52	42.19	44.72
	Total	8.55	0	10.15	22.52	42.19	44.72
UF 38 ppm-N	NH ₃ -N	9.21	0	0	0	0	0
	NO ₃ ⁻ -N	0	0	14.58	37.37	47.91	43.66
	Total	9.21	0	14.58	37.37	47.91	43.66
UF 50 ppm-N	NH ₃ -N	7.07	6.09	0	0	0	0
	NO ₃ ⁻ -N	0	0	18.06	42.50	40.98	35.23
	Total	7.07	6.09	18.06	42.50	40.98	35.23

Table 10. Total chlorophyll (mg/g fresh weight) for ryegrass plants grown in the sandy loam soil.

Growth Period	I	II	III	IV
Treatment Days	0-17	14-31	21-37	28-45
Control	1.53	1.37	1.17	1.31
Urea 25 ppm-N	1.54	1.36	1.22	1.38
Urea 38 ppm-N	1.59	1.40	1.26	1.40
Urea 50 ppm-N	1.62	1.49	1.28	1.40
Urea 25 ppm-N NI 0.25 ppm	1.56	1.44	1.24	1.32
Urea 25 ppm-N NI 0.50 ppm	1.69	1.40	1.22	1.38
Urea 25 ppm-N NI 1.0 ppm	1.86	1.47	1.16	1.36
Urea 38 ppm-N NI 0.25 ppm	1.56	1.44	1.41	1.45
Urea 38 ppm-N NI 0.50 ppm	1.76	1.40	1.33	1.36
Urea 38 ppm-N NI 1.0 ppm	1.72	1.52	1.40	1.49
Urea 50 ppm-N NI 0.25 ppm	1.67	1.44	1.44	1.41
Urea 50 ppm-N NI 0.50	1.82	1.43	1.35	1.42
Urea 50 ppm-N NI 1.0 ppm	1.77	1.50	1.28	1.45
UF 25 ppm-N	1.56	1.40	1.32	1.37
UF 38 ppm-N	1.61	1.46	1.23	1.37
UF 50 ppm-N	1.63	1.42	1.42	1.37

Table 11. Total chlorophyll (mg/g fresh weight) for ryegrass plants grown in the clay loam soil.

Growth Period	I	II	III	IV	
Treatment	Days	0-17	14-31	21-37	28-45
Control		1.54	1.61	1.35	1.40
Urea 25 ppm-N		1.52	1.61	1.32	1.42
Urea 38 ppm-N		1.52	1.67	1.37	1.37
Urea 50 ppm-N		1.62	1.70	1.41	1.39
Urea 25 ppm-N NI 0.25 ppm		1.59	1.61	1.34	1.37
Urea 25 ppm-N NI 0.50 ppm		1.59	1.64	1.36	1.33
Urea 25 ppm-N NI 1.0 ppm		1.60	1.72	1.32	1.25
Urea 38 ppm-N NI 0.25 ppm		1.64	1.58	1.30	1.22
Urea 38 ppm-N NI 0.50 ppm		1.69	1.65	1.24	1.20
Urea 38 ppm-N NI 1.0 ppm		1.65	1.69	1.33	1.32
Urea 50 ppm-N NI 0.25 ppm		1.65	1.68	1.35	1.40
Urea 50 ppm-N NI 0.50 ppm		1.73	1.75	1.35	1.29
Urea 50 ppm-N NI 1.0 ppm		1.69	1.80	1.36	1.29
UF 25 ppm-N		1.54	1.46	1.29	1.41
UF 38 ppm-N		1.52	1.60	1.48	1.40
UF 50 ppm-N		1.43	1.59	1.40	1.41

Figure 1. Recovery of $\text{NH}_3\text{-N}$ from the sandy loam soil at the three urea concentrations. The control has been subtracted from the treatment data.

Figure 2. Recovery of $\text{NO}_3\text{-N}$ from the sandy loam soil at the three urea concentrations. The control has been subtracted from the treatment data.

Figure 1.

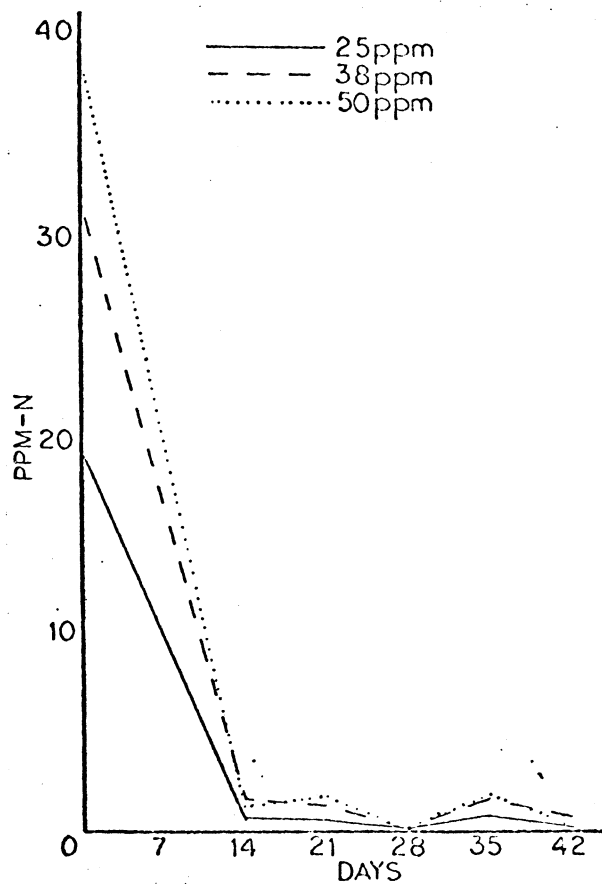


Figure 2.

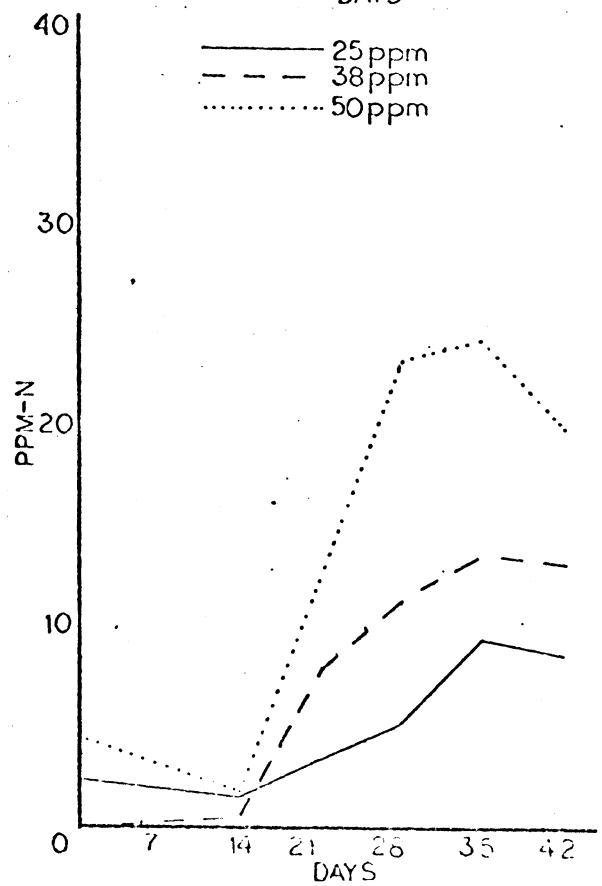


Figure 3. Recovery of $\text{NH}_3\text{-N}$ from the sandy loam soil at 25 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 4. Recovery of $\text{NO}_3\text{-N}$ from the sandy loam soil at 25 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 3.

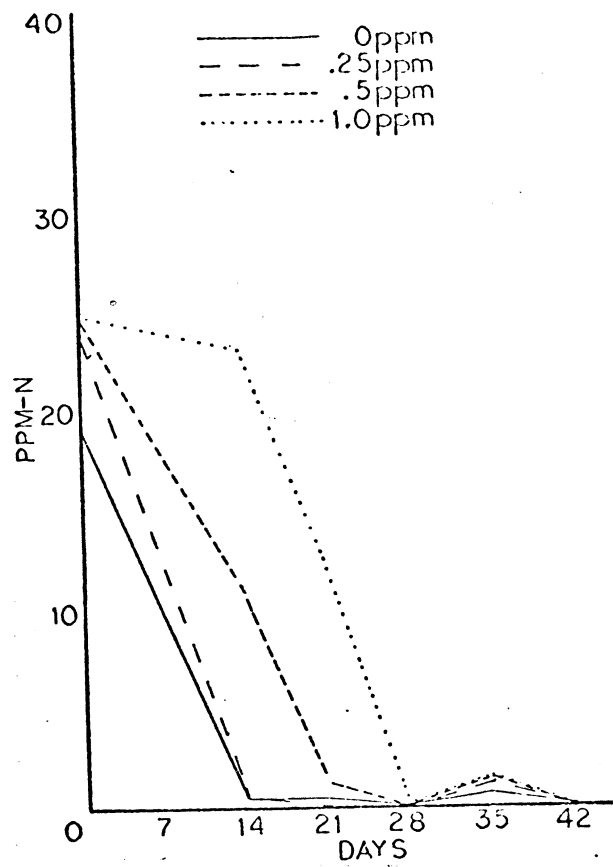


Figure 4.

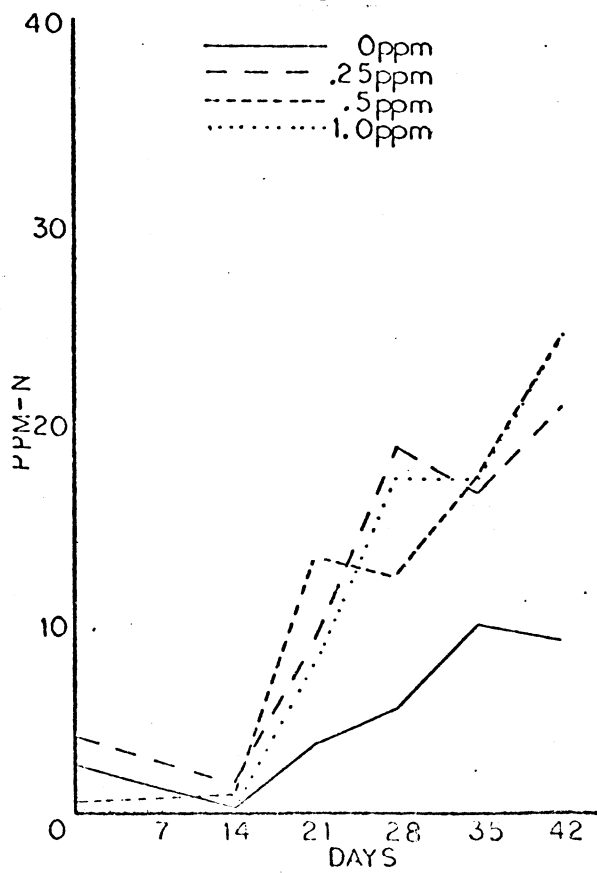


Figure 5. Recovery of $\text{NH}_3\text{-N}$ from the sandy loam soil at 38 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 6. Recovery of $\text{NO}_3\text{-N}$ from the sandy loam soil at 38 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 5.

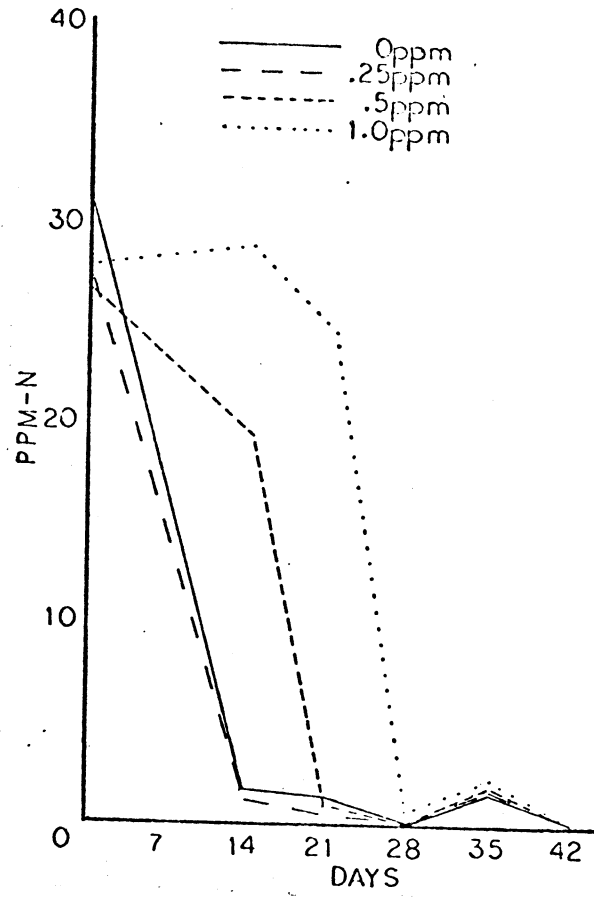


Figure 6.

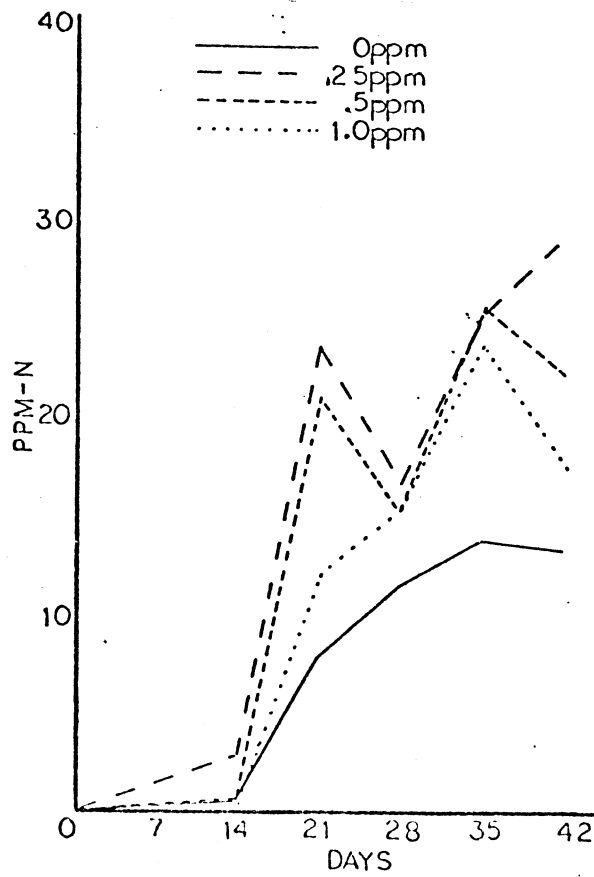


Figure 7. Recovery of $\text{NH}_3\text{-N}$ from the sandy loam soil at 50 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 8. Recovery of $\text{NO}_3\text{-N}$ from the sandy loam soil at 50 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 7.

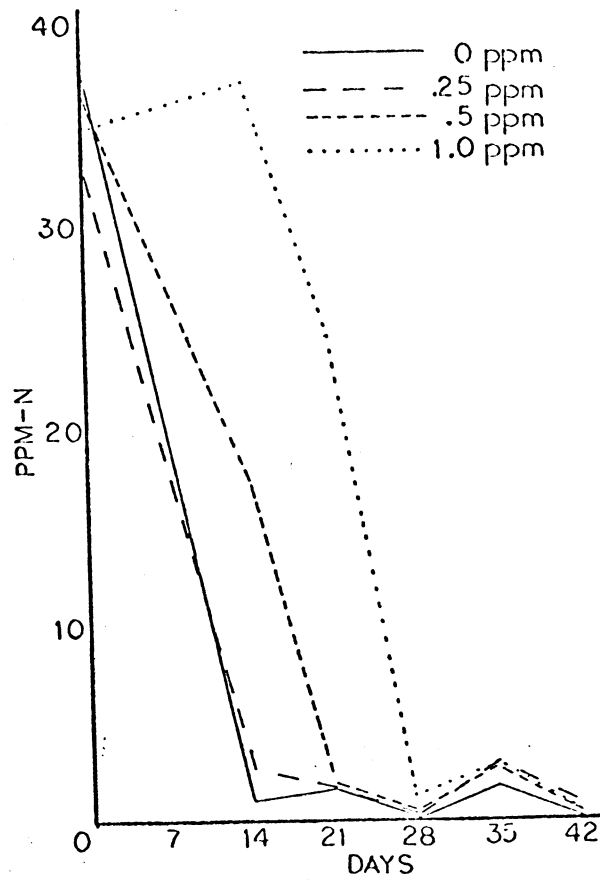


Figure 8.

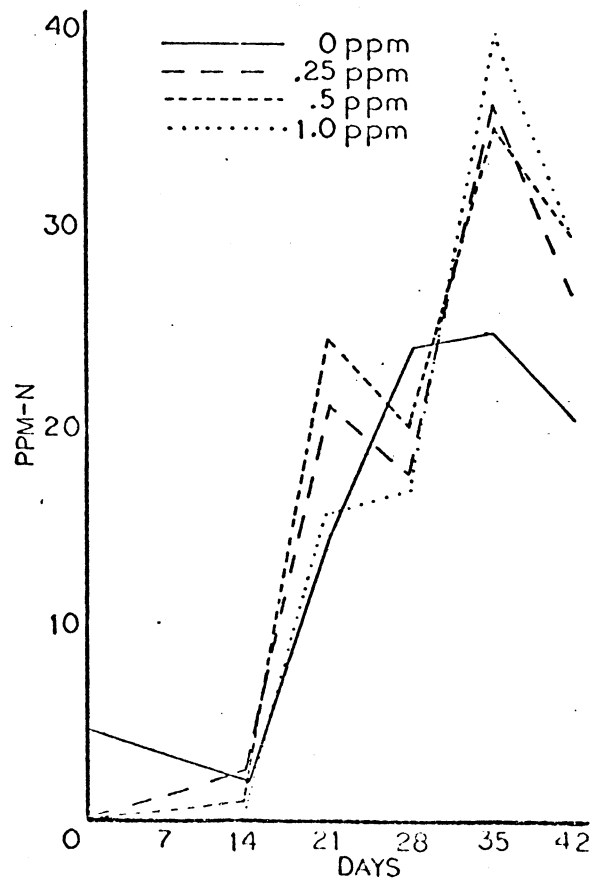


Figure 9. Recovery of $\text{NH}_3\text{-N}$ from the sandy loam soil at the three ureaformaldehyde concentrations. The control has been subtracted from the treatment data.

Figure 10. Recovery of $\text{NO}_3\text{-N}$ from the sandy loam soil at the three ureaformaldehyde concentrations. The control has been subtracted from the treatment data.

Figure 9.

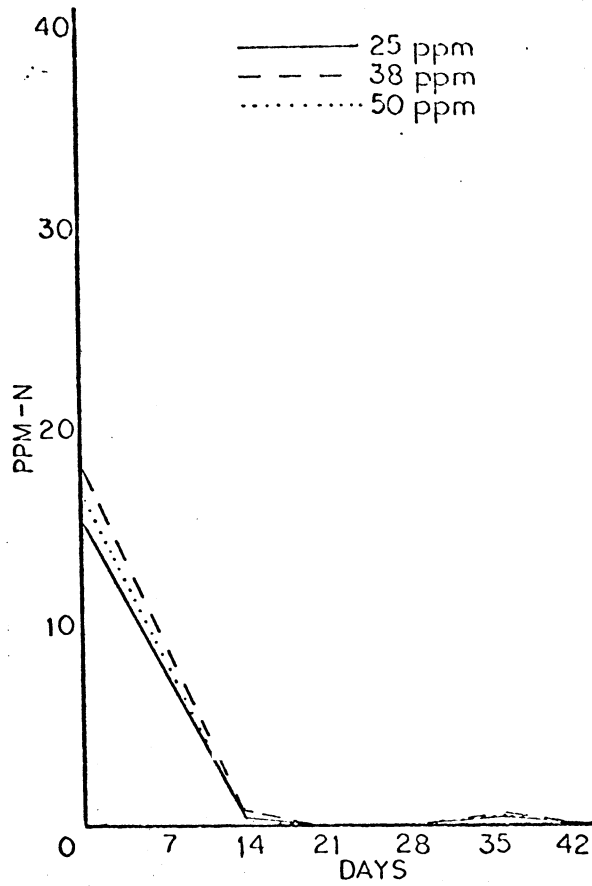


Figure 10.

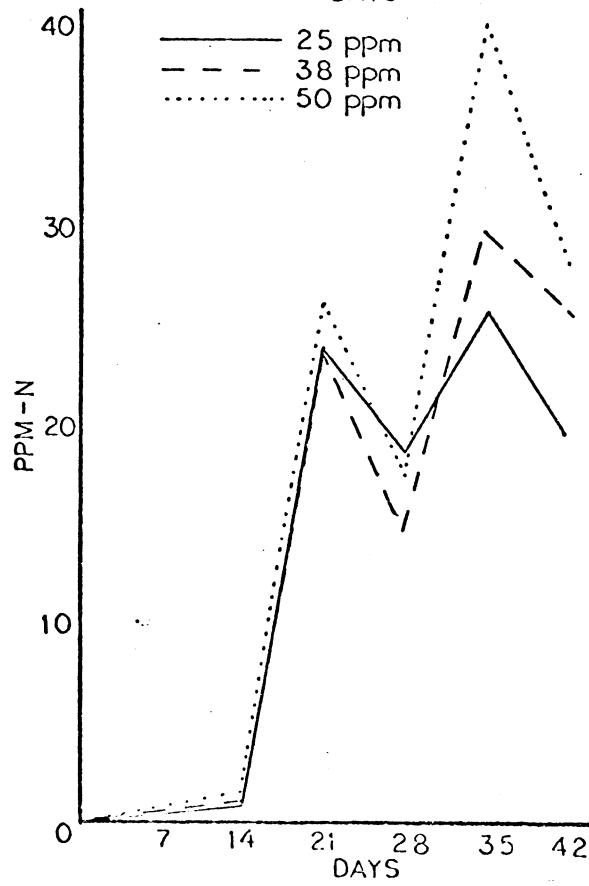


Figure 11. Recovery of $\text{NH}_3\text{-N}$ from the clay loam soil at the three urea concentrations. The control has been subtracted from the treatment data.

Figure 12. Recovery of $\text{NO}_3\text{-N}$ from the clay loam soil at the three urea concentrations. The control has been subtracted from the treatment data.

Figure 11.

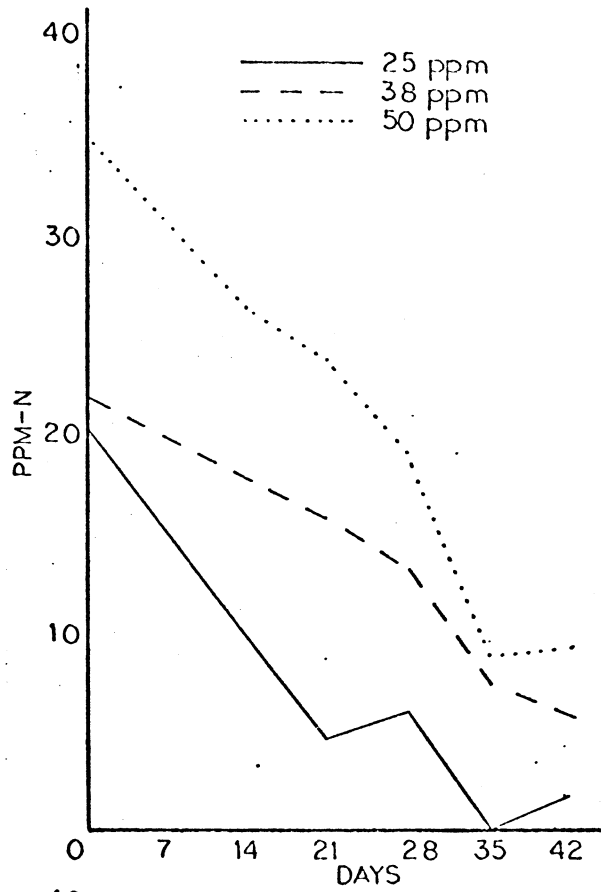


Figure 12.

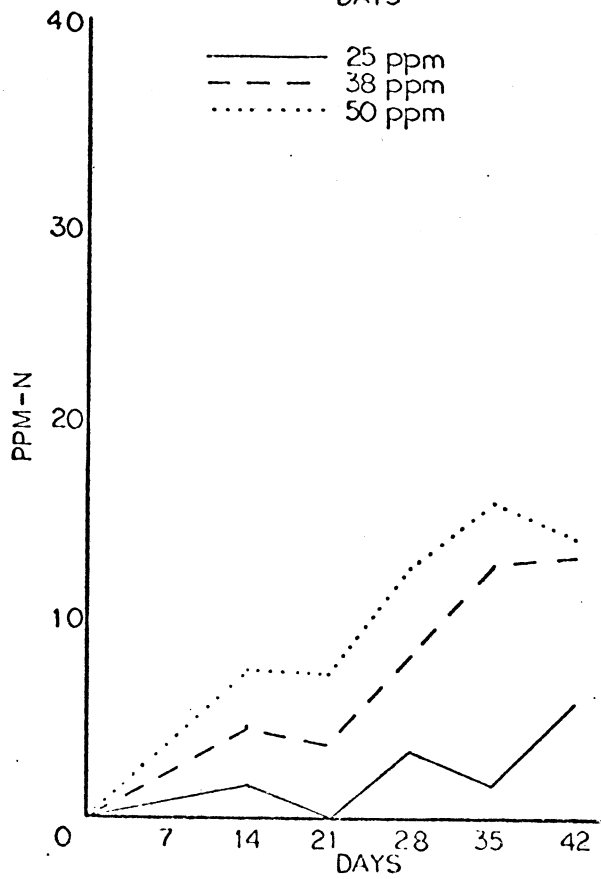


Figure 13. Recovery of $\text{NH}_3\text{-N}$ from the clay loam soil at 25 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 14. Recovery of $\text{NO}_3\text{-N}$ from the clay loam soil at 25 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 13.

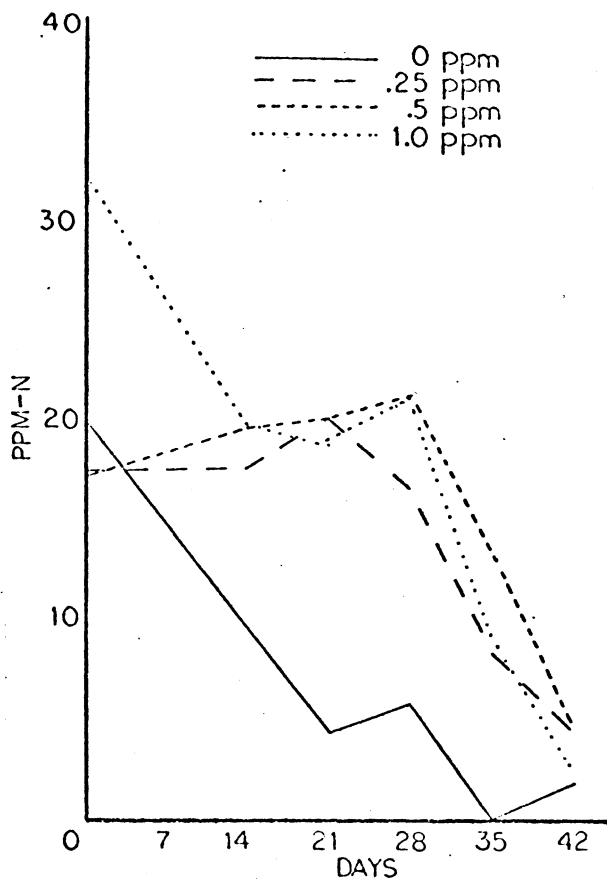


Figure 14.

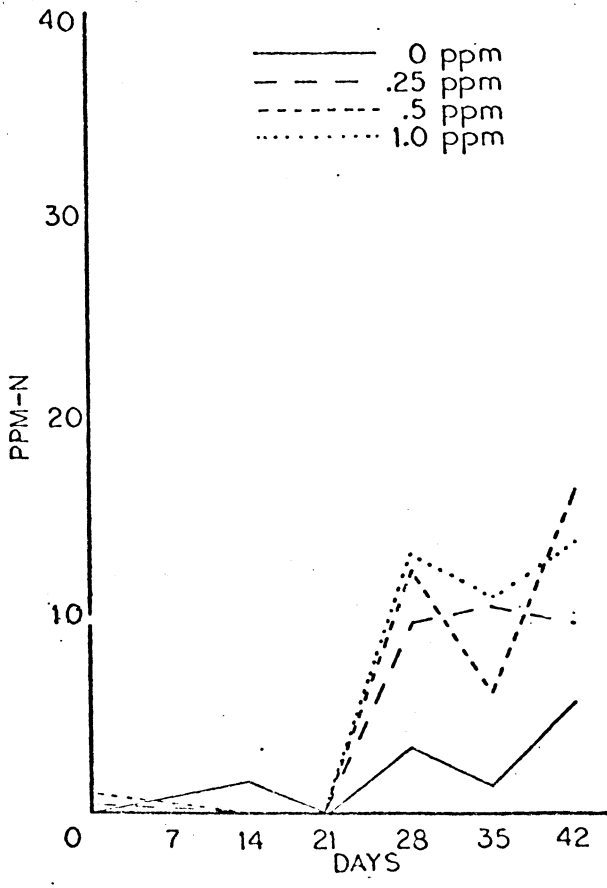


Figure 15. Recovery of $\text{NH}_3\text{-N}$ from the clay loam soil at 38 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 16. Recovery of $\text{NO}_3\text{-N}$ from the clay loam soil at 38 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 15.

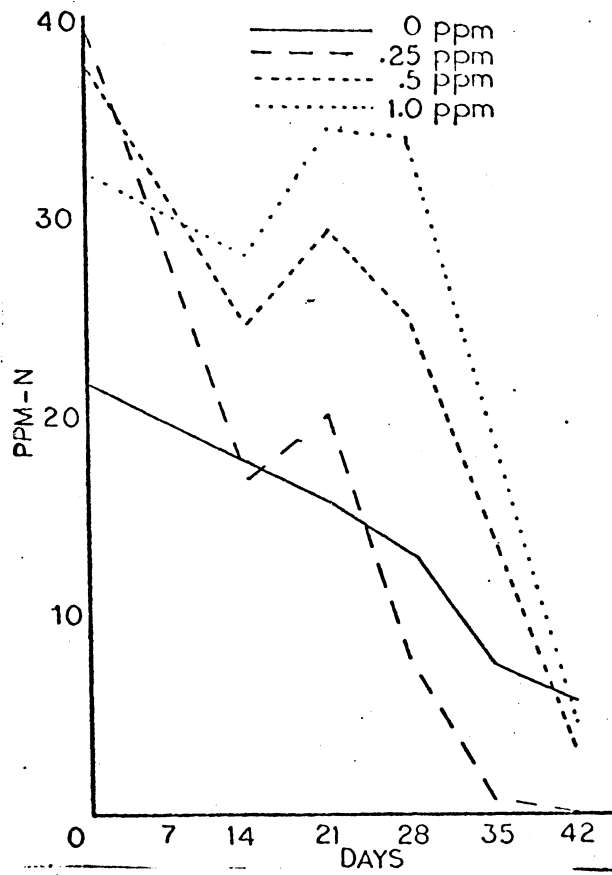


Figure 16.

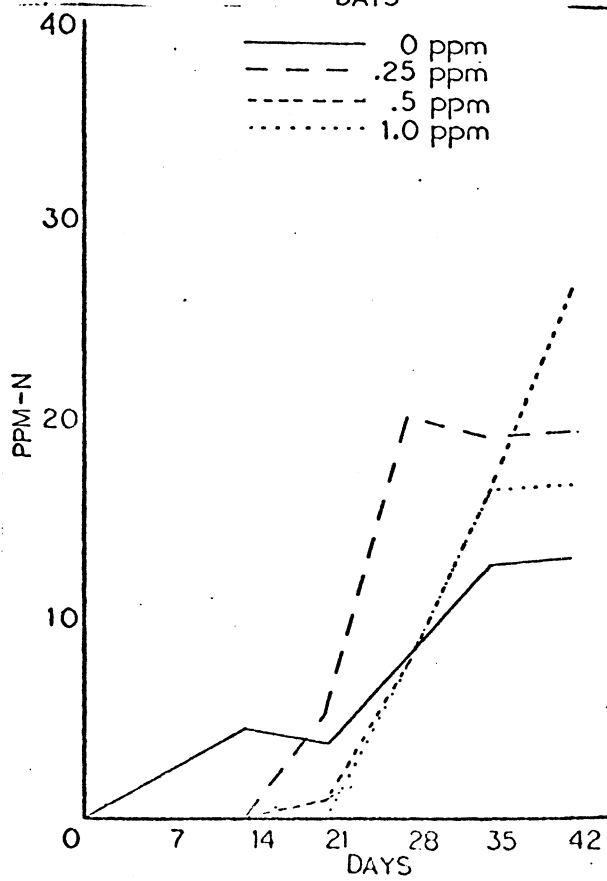


Figure 17. Recovery of $\text{NH}_3\text{-N}$ from the clay loam soil at 50 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 18. Recovery of $\text{NO}_3\text{-N}$ from the clay loam soil at 50 ppm-N urea in the presence of three concentrations of N-Serve. The control has been subtracted from the treatment data.

Figure 17.

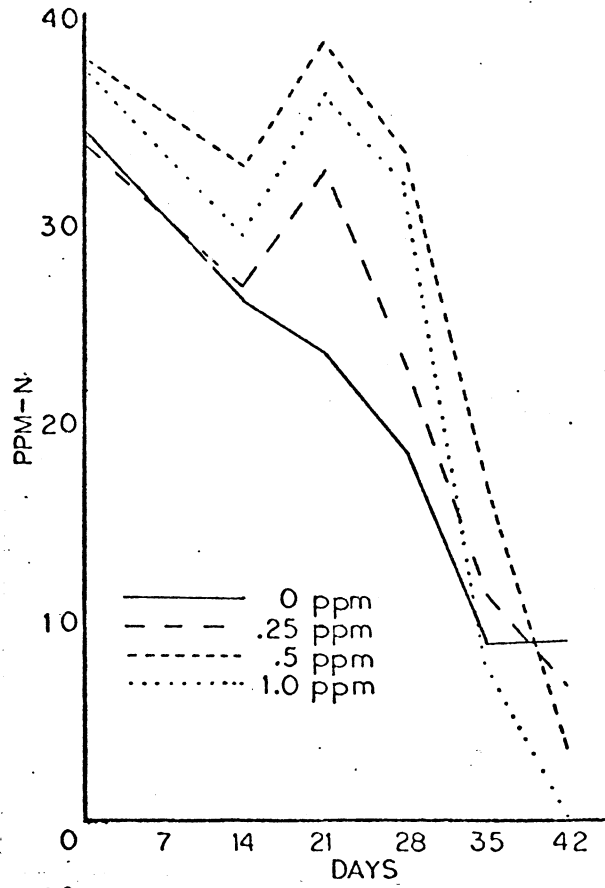


Figure 18.

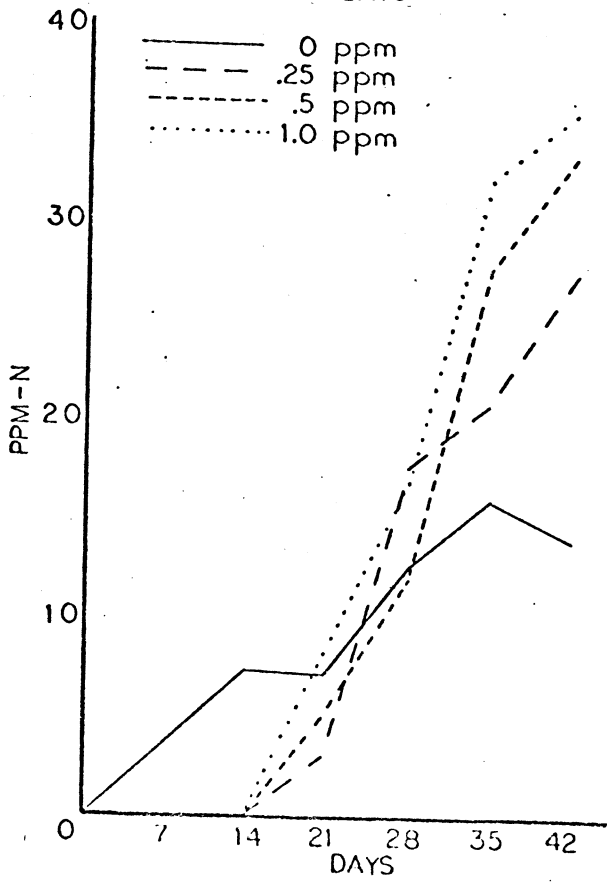


Figure 19. Recovery of $\text{NH}_3\text{-N}$ from the clay loam soil at the three ureaformaldehyde concentrations. The control has been subtracted from the treatment data.

Figure 20. Recovery of $\text{NO}_3\text{-N}$ from the clay loam soil at the three ureaformaldehyde concentrations. The control has been subtracted from the treatment data.

Figure 19.

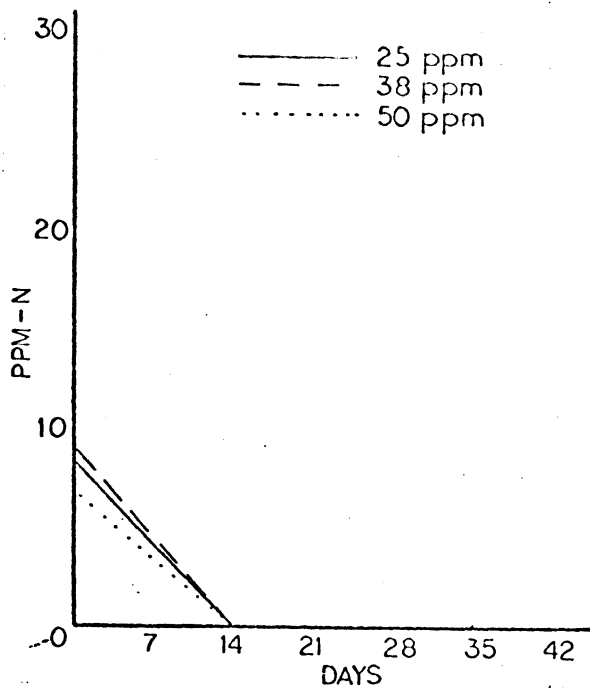
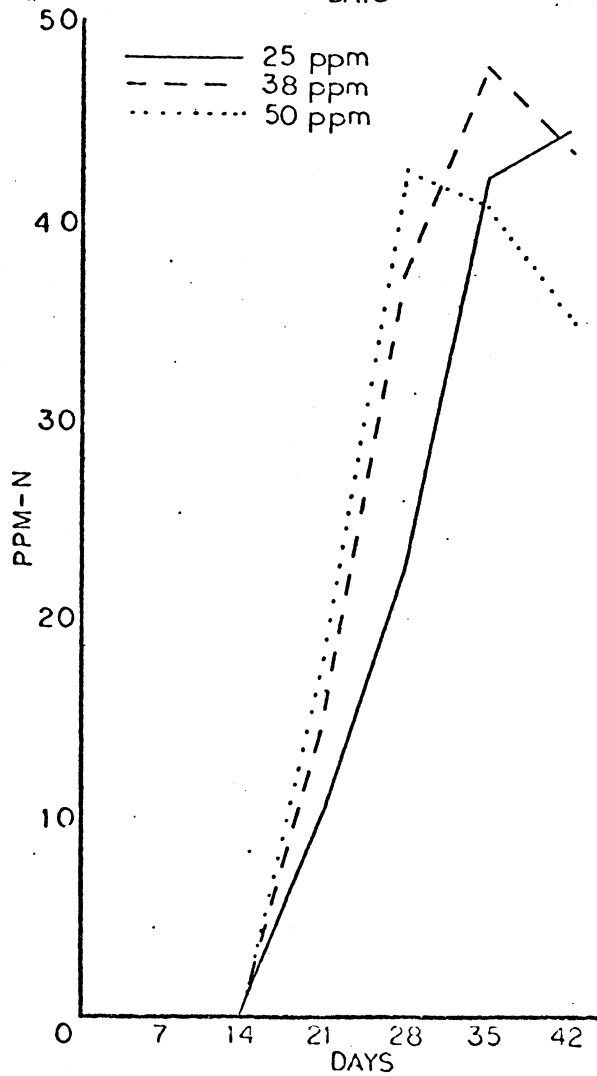


Figure 20.



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