



Influence of NH_3 from ammonium phosphate fertilizers on germination, seedling growth, and small plant yield of wheat (*Triticum aestivum* L.)
by Chaitat Pairintra

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY in Crop and Soil Science
Montana State University
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Abstract:

Influences of NH_3 from ammonium phosphate fertilizers on germination, seedling growth, and small plant yield of wheat (*Triticum aestivum* L.) were evaluated in laboratory, growth chamber, greenhouse, and field experiments. Measurements of NH_3 were made using techniques of diffusion from the soil-fertilizer system and distillation of NH_3 from the seed-plant system.

A "Diffusion Can" was designed for quantitative measurement of NH_3 production from the ammonium phosphates—mono (MAP, 11-48-0), di (DAP, 18-46-0), poly (APP, 15-62-0), and urea (UAP, 24-42-0)—upon reaction with 10 soils varying in moisture from 10 to 25% and in CaCO_3 contents from zero to 12%. Total amount of NH_3 produced from fertilizer reacting 6 days was in the order of magnitude: $\text{UAP} \gg \text{DAP} \gg \text{MAP} > \text{APP}$, or the ratio of 18 : 4.5 : 1.5 : 1. This relationship was generally true for any soil, but the absolute values increased directly with % CaCO_3 in the soil and inversely with soil moisture. Averaging all soils, the total 6-day NH_3 production was in the soil moisture order of 10% > 15% > 20%.

Ammonia absorption by plants was measured directly from the seeds or seedlings by a "Distillation Technique", and indirectly by differences between "Soil-Fertilizer" mixed and "Soil-Fertilizer-Seed" systems. The patterns of NH_3 accumulation by seeds or seedlings were governed by the kinds and rates of fertilizer application, soil moisture, CaCO_3 content, and the stages of plant germination or growth.

Dry weight of wheat seedlings was less when germinating seeds, with radicles emerged, were exposed to NH_3 for one day than when seeds were treated with NH_3 one day after moistening.

Absorption of NH_3 by seedlings was directly related to NH_3 production from fertilizer-soil reactions. Maximum absorption occurred in day 3 for 10% CaCO_3 soil and day 2 for 0% CaCO_3 , but the latter was a very low absorption except for UAP. Ammonia concentration in seeds or seedlings in the range 0.3 to 0.5 ppm- NH_3 inhibited seedling growth, and symptoms of NH_3 injury were evident. Radicles had a brown color as a "burnt off" appearance and coleoptiles were stunted. Germination of wheat seeds was completely prevented when the concentration of NH_3 in seeds reached about 0.8 ppm- NH_3 .

Plant yield results from growth chamber studies on 0% CaCO_3 soil indicated the same responses to fertilizers for roots as for tops with the fertilizers in the order: $\text{APP} > \text{MAP} > \text{DAP} > \text{UAP}$. On the 10% CaCO_3 soil the order was $\text{APP} > \text{MAP} > \text{DAP} > \text{UAP}$, and growth of roots for DAP and UAP increased relatively little from 6 to 12 days in contrast with APP and MAP. In all cases, plant growth was inversely related to concentration of NH_3 in plants. Ammonia measured by microdiffusion from soil samples taken from field experiments having fertilizer banded with wheat seed was directly related to reduction in numbers of crowns and stems and inversely related to dry weights of plants at the stem elongation stage of growth.

Using information developed in this research 4 ammonium phosphate fertilizers reacting with soils

varying in CaCO₃ content can be arranged according to NH₃ production. If greater than 0.5 µg NH₃/100g soil measured by the diffusion can technique is considered potentially hazardous for banding fertilizers with seeds, the fertilizers and allowable soil CaCO₃ percentages before serious seedling damage occurs are as follows: APP, 12% CaCO₃; MAP, 10.5% CaCO₃; DAP, 3.5% CaCO₃; and UAP exceeds the limit at 0% CaCO₃.

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ABSTRACT

Influences of NH_3 from ammonium phosphate fertilizers on germination, seedling growth, and small plant yield of wheat (*Triticum aestivum* L.) were evaluated in laboratory, growth chamber, greenhouse, and field experiments. Measurements of NH_3 were made using techniques of diffusion from the soil-fertilizer system and distillation of NH_3 from the seed-plant system.

A "Diffusion Can" was designed for quantitative measurement of NH_3 production from the ammonium phosphates--mono (MAP, 11-48-0), di (DAP, 18-46-0), poly (APP, 15-62-0), and urea (UAP, 24-42-0)--upon reaction with 10 soils varying in moisture from 10 to 25% and in CaCO_3 contents from zero to 12%. Total amount of NH_3 produced from fertilizer reacting 6 days was in the order of magnitude: $\text{UAP} \gg \gg \gg \text{DAP} \gg \gg \text{MAP} > \text{APP}$, or the ratio of 18 : 4.5 : 1.5 : 1. This relationship was generally true for any soil, but the absolute values increased directly with % CaCO_3 in the soil and inversely with soil moisture. Averaging all soils, the total 6-day NH_3 production was in the soil moisture order of 10% > 15% > 20%.

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Absorption of NH_3 by seedlings was directly related to NH_3 production from fertilizer-soil reactions. Maximum absorption occurred in day 3 for 10% CaCO_3 soil and day 2 for 0% CaCO_3 , but the latter was a very low absorption except for UAP. Ammonia concentration in seeds or seedlings in the range 0.3 to 0.5 ppm- NH_3 inhibited seedling growth, and symptoms of NH_3 injury were evident. Radicles had a brown color as a "burnt off" appearance and coleoptiles were stunted. Germination of wheat seeds was completely prevented when the concentration of NH_3 in seeds reached about 0.8 ppm- NH_3 .

Plant yield results from growth chamber studies on 0% CaCO_3 soil indicated the same responses to fertilizers for roots as for tops

with the fertilizers in the order: $APP \geq MAP > DAP > UAP$. On the 10% $CaCO_3$ soil the order was $APP > MAP > DAP > UAP$, and growth of roots for DAP and UAP increased relatively little from 6 to 12 days in contrast with APP and MAP. In all cases, plant growth was inversely related to concentration of NH_3 in plants. Ammonia measured by microdiffusion from soil samples taken from field experiments having fertilizer banded with wheat seed was directly related to reduction in numbers of crowns and stems and inversely related to dry weights of plants at the stem elongation stage of growth.

Using information developed in this research 4 ammonium phosphate fertilizers reacting with soils varying in $CaCO_3$ content can be arranged according to NH_3 production. If greater than $0.5 \mu g NH_3/100g$ soil measured by the diffusion can technique is considered potentially hazardous for banding fertilizers with seeds, the fertilizers and allowable soil $CaCO_3$ percentages before serious seedling damage occurs are as follows: APP, 12% $CaCO_3$; MAP, 10.5% $CaCO_3$; DAP, 3.5% $CaCO_3$; and UAP exceeds the limit at 0% $CaCO_3$.

INTRODUCTION

Fertilizing has long been proved to be an effective method to increase crop production. In spite of the advantages of fertilizer application, increased emphasis is now being placed on the problem of plant damage resulting from improper fertilizer use. Nitrogenous fertilizers, one of the greatest consumptive use, when applied to soils under certain conditions, can release free NH_3 . This ammonia may injure small plants. Detrimental effects as a consequence of NH_3 toxicity would be expected to be associated with the concentration of NH_3 produced and the various susceptible stages of plant development (Smith et al., 1970).¹ Therefore, the questions of how NH_3 is released, how it produces toxic effects on germination and seedling growth and/or how it influences yield components of plants, become matters of practical as well as theoretical interest.

It is hypothesized that, upon the hydrolysis of ammonium phosphate fertilizers in the soil, NH_3 is released and it is the major factor in producing toxic symptoms to germination and seedlings, and it may influence yield components of wheat. Therefore, to achieve a systematic interrelation of facts, the investigations of this hypothesis were conducted intensively in field, greenhouse, and laboratory experiments. Specific objectives and experimental procedures pertinent to

¹Smith, C. M., E. O. Skogley, and C. Pairintra. 1970. Farm test demonstrations. Ann. Report to TVA (Unpublished).

the different types of experiments are illustrated under each later section.

LITERATURE REVIEW

Tisdale and Nelson (1966) wrote that progress in agriculture depends on research of a high caliber. For every problem solved by the scientist today, many more are raised. Agricultural scientists must delve into questions of a fundamental nature, questions that deal more with the WHY of things than with the WHAT.

It is, therefore, the purpose of this review to bring together some of the pertinent findings so that causes and effects can be evaluated and some possible corrective addition may be established.

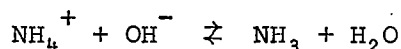
Because ammonia volatilization and toxicity vary depending upon chemical composition of fertilizer and properties of soils, this presentation is, therefore, divided into three distinct parts: (I) Theory, (II) Ammonia Volatilization, and (III) Ammonia Toxicity. All of these aspects are discussed in relation to fertilizer and soil properties.

I. Theory

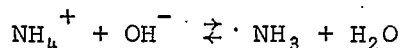
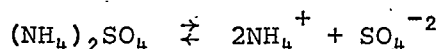
Bennett and Adams (1970) stated recently that ammoniacal-N loss from soil and toxicity to seedlings are parallel manifestations of the same phenomenon. Both depended upon the $\text{NH}_3(\text{aq})$ concentration in the soil solution and are thus governed by the same chemical equilibria. Failure to adequately consider all equilibria have prevented previous investigators from establishing general applicable

quantitative parameters for ammonia losses or toxicity (Blanchar, 1967; Du Plessis and Kroontje, 1964; Ernst and Massey, 1960; Larsen and Gunary, 1962; Megie et al., 1967; Wahhab et al., 1957).

Mechanisms of NH_3 volatilization from soil by chemical reaction have been postulated and almost all systems are pH-dependent. Du Plessis and Kroontje (1964) investigated the relationship between pH and ammonia equilibria in soil and suggested that NH_3 volatilized from acid soils was due to the equilibrium.



Also, there has been a proposal (Wahhab et al., 1957) that NH_3 losses occurred from slightly acid soils to which $(\text{NH}_4)_2\text{SO}_4$ was added, and could be due to the equilibrium of the following nature:



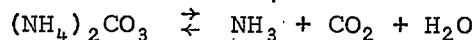
That the effective hydroxyl concentration in such a case would depend on the pH of the system. This postulation was given some support by Ernst and Massey (1960) who stated that liming would cause an increase in soil pH and thus favor the increase in the activity of OH^- . This would encourage the shift in the reaction to the right and increase the volatilization of NH_3 .

Larsen and Gunary (1962) considered that losses of NH_3 from fertilizers applied on alkaline or calcareous soils depend on the

equilibrium:

$$P_{\text{NH}_3} = K[\text{NH}_4]/([\text{Ca}^{2+}]P_{\text{CO}_2})^{1/2}$$

where K is a constant equal to $K_{\text{NH}_4}/K_{\text{CaCO}_3}$ and P_{CO_2} is partial pressure of CO_2 . The mechanism affecting the right side is to reduce $[\text{NH}_4^+]$, thus increasing NH_3 volatilization loss. They suggested NH_4^+ can be removed from the system by precipitation of insoluble calcium ammonium phosphates. Terman and Hunt (1964) reported that the $(\text{NH}_4)_2\text{CO}_3$ which is formed from chemical reactions of fertilizers and CaCO_3 in the soil is unstable and decomposes easily due to the equilibrium:



II. Ammonia Volatilization

It has been established that NH_3 may be volatilized readily from soils under a number of conditions and that many factors are involved. Several investigators studied factors affecting NH_3 volatilization losses from nitrogen fertilizer carriers. Mortland (1958) in reviewing work on the reaction of NH_3 in soils listed soil moisture, texture, pH, organic matter, placement, and soil tilth as the factors affecting sorption and loss of NH_3 in soils.

Recently, Pesek et al. (1971) stated that the likelihood of ammonia losses from surface applications is dependent primarily on the chemical nature of the fertilizer material and the pH and other

properties of the soil. They concluded that the modifying influences include soil water content, temperature, surface roughness and residue, air movement, presence of carbonates, granule size of fertilizer and time elapsed between application and the next rainfall, irrigation or before incorporation by tillage.

Stanley and Smith (1955) and Tseng and Wang (1967) observed in the laboratory that losses of ammonia from sandy soil were greater than from finer textures such as silt loam and clay. However, Jenny et al. (1945) reported that ammonia retention was a function of soil texture.

Martin and Chapman (1951) conducted a laboratory experiment on volatilization of ammonia from surface-fertilized soils. The experimental results indicated that percentages of $\text{NH}_3\text{-N}$ lost were in the order of magnitude: $\text{NH}_4\text{OH} > (\text{NH}_4)_2\text{SO}_4 > \text{Urea} > \text{NH}_4\text{NO}_3 > \text{NaNO}_3 = \text{check}$. They reasoned that NH_4OH application raised the pH of the surface of acid soils to the alkaline range and thus permitted greater $\text{NH}_3\text{-N}$ volatilization losses. Increasing rates of application also increased the rates of $\text{NH}_3\text{-N}$ volatilization losses in acid soils but not for alkaline soils.

In laboratory studies, Kresge and Satchell (1960) compared the amount of NH_3 lost by volatilization from several fertilizers. Experimental results showed that the NH_3 volatilization was in the order of magnitude: $\text{Urea} > \text{Ca}(\text{CN})_2 > (\text{NH}_4)_2\text{SO}_4 > \text{NH}_4\text{NO}_3$. The rates of NH_3 losses

were significantly different among fertilizers and were increased as the rates of application increased. In a series of field, greenhouse, and laboratory experiments, Meyer et al. (1961) obtained the same trend of NH_3 volatilization losses.

Regardless of pH and soil type, the magnitudes of NH_3 volatilization presented by Larsen and Gunary (1962) were in the order:

$(\text{NH}_4)_2\text{SO}_4 > (\text{NH}_4)_2\text{HPO}_4 = \text{NH}_4\text{H}_2\text{PO}_4 = \text{NH}_4\text{NO}_3$, whereas Terman and Hunt (1964) reported that NH_3 volatilization losses were in the order: Urea > Urea ammonium phosphate > $(\text{NH}_4)_2\text{HPO}_4 > (\text{NH}_4)_2\text{SO}_4 > \text{Ammonium polyphosphate} = \text{NH}_4\text{NO}_3 > \text{NH}_4\text{H}_2\text{PO}_4$.

Jewitt (1942) reported that when $(\text{NH}_4)_2\text{SO}_4$ was applied to soils, considerable amounts of NH_3 were lost through volatilization and these losses were influenced by the rate of fertilizer application. A recent study (Mills et al., 1970) reported similar experimental findings.

Mitsui (1954) and Terman and Hunt (1964) reported a marked decrease in NH_3 losses from urea mixed with the soil compared to surface application. Overrein and Moe (1967) added that NH_3 volatilization rates were inversely proportional to the depth of urea application. Steenbjerg (1944) noted that in 4 weeks the losses from NH_3 from surface application of urea ranged from 5-60%, but if the placement of fertilizer was at 6 cm depth, there was no loss of NH_3 .

Ernst and Massey (1960) stated that NH_3 volatilization from soil was essentially the same when urea was topdressed or mixed with the top 1/4 inch of soil. However, the data of cumulative losses of NH_3 from urea and $(\text{NH}_4)_2\text{SO}_4$ reported by Gasser (1964) indicated that NH_3 volatilization losses were influenced by placement and varied depending on the kind of fertilizers.

Martin and Chapman (1951) reported that CEC (cation exchange capacity) of the soil was important in determining losses of ammonia. Gasser (1964) stated that effects of soils on ammonia losses depend largely on base exchange capacity. He said that property was the most likely one to be related to the ability of the soil to retain ammonium nitrogen and ammonia. The results indicated that ammonia loss decreased as the base exchange capacity increased. Volk (1959) illustrated the effect of CEC as it influenced loss of ammonia by volatilization from surface application of urea and $(\text{NH}_4)_2\text{SO}_4$. During the first week, there was a significant decrease in loss from the application of urea but not for $(\text{NH}_4)_2\text{SO}_4$, and the losses were directly related to the increases in CEC of the 11 acid soils.

Pertaining to reaction of ammonia with clay minerals such as bentonite, Mortland (1958) concluded that the effect of exchangeable cations on ammonia desorption was found to follow the order $\text{H}^+ > \text{Ca}^{+2} > \text{Na}^+ > \text{K}^+$. He stated that the fixation of K^+ by bentonite particularly reduced the sorption of ammonia. This is in agreement with the

work by Martin and Chapman (1951) who found more NH_3 volatilization losses when the exchangeable cation was Na^+ or K^+ than when it was Ca^{+2} or Mg^{+2} . They attributed the effect to the high pH of the Na- and K-saturated soils. Also, other investigators have shown CEC to have a very marked effect on the amount of NH_3 volatilization loss (Brown and Bartholomew, 1962; Ernst and Massey, 1960).

A recent investigation by Rolston et al. (1972) on desorption of ammonia from soil during ion displacement studies indicated that a moist soil has a greater capacity for ammonia desorption than a dry one. In Russia, Lyakh (1972) reported that addition of NPK fertilizers with decreasing soil moisture increased NH_3 losses.

In reviewing literature on ammonia reaction, Mortland (1958) concluded that sorption of ammonia in the soil was influenced by soil moisture. He stated that since ammonia will dissolve in water, the concentration would depend on the partial pressure of NH_3 . Any ammonia that does dissolve in the soil water is in transitory condition; it either will react chemically with organic matter or will volatilize into the air if the partial pressure of NH_3 is exceeded. Stanley and Smith (1955) explained that losses of ammonia from the wet soils were the result of upward movement and subsequent evaporation of water containing dissolved ammonia, whereas from dry soils the losses of ammonia resulted from gas flow out of the soil as a part of gas vapor pressure.

Wetting and drying caused greater losses of NH_3 than maintaining the soil at constant moisture content (Jones, 1932). Ernst and Massey (1960) reported that some NH_3 volatilization occurred without a concurrent drying process in the soil, but greater volatilization occurred when moisture was lost from the soil. When the soil became dry after 4 to 5 days of aeration, NH_3 volatilization was markedly decreased, presumably because hydrolysis of urea was retarded due to the lack of moisture. Therefore, they concluded that NH_3 volatilization was directly related to initial soil moisture content. In contrast, Jewitt (1942) found the loss of ammonia from $(\text{NH}_4)_2\text{SO}_4$ fertilized soils to be dependent on a drying process in the soil but not on the initial soil moisture content.

Martin and Chapman (1951) observed no volatilization of ammonia when moist air was passed over N-fertilized soil (NH_4^+ forms), but loss of ammonia did occur when the samples were aerated with dry air and thus were losing moisture. Meyer et al. (1961) concluded that in favorably moist soils, volatilization of NH_3 took place rapidly in the first few days after application, then tapered off to an insignificant rate in the second week.

Several investigators (Broadbent et al., 1958; Doak, 1952; Fisher and Parks, 1958) reported that urea is readily soluble in water, and the dissolved urea is hydrolyzed to ammonium carbonate by soil bacteria and enzymes. The rate of hydrolysis apparently varies a great

deal among soils, but is temperature-dependent in a particular soil. Overrein and Moe (1967) observed that the rate of urea hydrolysis was shown to be directly proportional to the rate of urea application when soils were incubated at 28°C.

Martin and Chapman (1951) stated that if the soil solution is alkaline in nature, then parts of ammonia will be present as hydrated ammonia, ammonium hydroxide, ammonium bicarbonate or carbonate, or both, depending on the alkalinity, concentration, and other factors. They found that in aqueous solution of these compounds, the NH_3 and water have their own partial vapor pressures and evaporate together in varying proportions depending on the concentration and character of the NH_3 containing solutions. Therefore, they concluded that increasing the amounts of ammonium nitrogen applied to alkaline soil tended to increase the total quantity of ammonia loss but did not appreciably affect the total percent loss.

Terman and Hunt (1964) stated that the differences in ammonia losses among nitrogenous fertilizers can be explained largely in terms of reaction of certain acid radicals of ammonium salts with calcium compounds in the soils. They illustrated the overall reactions which presumably occurred in limed acid or naturally calcareous soils and concluded that ammonium carbonate which is formed by hydrolysis processes is unstable and decomposes easily into NH_3 , CO_2 and H_2O . Bates and Pinching (1950) demonstrated that when ammonium carbonate is

formed, and if the pH of the system is above 7, the concentration of NH_3 increases while NH_4^+ decreases.

III. Ammonia Toxicity

The detrimental effects of ammoniacal-N from fertilizers on germination and seedling growth of plants have received attention in recent years (Bennett and Adams, 1970; Colliver and Welch, 1970; Guttay, 1957; Warren, 1962). Although ammonia is known to be toxic to most forms of plant and animal life, some of the specific toxicity mechanism and physiological effects of plant have not been adequately explained.

Vines and Wedding (1960) studied the mechanism of ammonia toxicity to intact plants and postulated that the site of ammonia toxicity to plants is located in the electron transport system, especially the $\text{DPNH} \rightarrow \text{DPN}$ reaction. Warren (1962) pointed out that the cell membranes were relatively impermeable to NH_4^+ , whereas NH_3 passed tissue barriers with ease. Therefore, he concluded that toxicity depended largely upon the NH_3 which entered the organism and cell. Along the same principle, Stuart and Haddock (1968) reported that $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{CO}_3$ or gaseous NH_3 inhibited water uptake in sugarbeet roots whenever the pH was sufficiently high. They suggested that the site of inhibition lies within the root epidermis.

Strogonov (1964) stated that the toxic effect of gaseous NH_3 is expressed by a change in the pH of the cell sap, by deformation of chloroplast, and by a destruction of the cell protein. Several investigators reported that NH_3 inhibits both photosynthetic (Kramer, 1955) and oxidative (Racker, 1961) formations. Murata (1969) concluded that heavy application of nitrogen very likely deteriorate the photosynthesis-respiration balance of the crop stand. He added that this is because if nitrogen supply is too abundant in comparison with the rate of carbohydrate production, the plants will sooner or later be depleted of carbohydrate reserve. Therefore, such plants face the danger of NH_3 toxicity.

Although there is recognition of ammonia release that is toxic to plants, there is no general agreement among researchers defining applicable quantitative parameters and concentrations for ammonia toxicity. Furthermore, it has been a subject of controversy.

Blanchar (1967) proposed a method to determine partial pressure of NH_3 in soil air. The partial pressure of NH_3 (P_{NH_3}) in a closed soil system was measured by expelling it from a collapsible plastic bottle into dilute HCl. The P_{NH_3} in the soil air was calculated from the ideal gas law. Since concentration of $\text{NH}_3\text{-N}$ was governed by different pH levels, Megie et al. (1967) concluded that the toxicity was pH-dependent and the toxicity was attributed to ammonia.

Sample (1963)¹ presented a comprehensive literature review on the effect of fertilizer materials on the germination of seeds. He concluded that free NH_3 gas is the main toxic substance.

Many workers have reported injury or delay in germination and emergence of various crops as a result of fertilizers, especially $(\text{NH}_4)_2\text{HPO}_4$. Hood and Ensminger (1964) reported that the detrimental effect of $(\text{NH}_4)_2\text{HPO}_4$ was not caused by osmotic effect, or by release of free ammonia alone, or by ammonium or phosphate ions per se. They suggested that $(\text{NH}_4)_2\text{HPO}_4$ might adversely affect Mg availability in the seed resulting in reduced enzymatic activity. In studying the mechanism of ammonium phosphate injury to seeds, Ensminger et al. (1965) concluded that germination injury from $(\text{NH}_4)_2\text{HPO}_4$ appeared to be largely due to the inactivation of Mg in seeds.

A recent investigation (Weir et al., 1972) illustrated that ammoniacal-N exists in more than one form in the soil solution; $\text{NH}_4^+\text{-N}$ and $\text{NH}_3\text{-N}$. Their experimental results indicated that plants responded similarly to NH_3 and NH_4^+ . Growth was reduced as the NH_3 concentration increased to an optimum level of 17 ppm of $\text{NH}_4^+\text{-N}$ for radish and 36 ppm for lettuce. Vines and Wedding (1960) reported, however, that the nonionized ammonia (NH_4^+) and gaseous ammonia (NH_3) inhibited

¹Sample, E. C. 1963. The effect of fertilizer materials on the germination of seeds: A literature review. TVA Report, 1963 (Unpublished).

respiration in concentrations of these two forms of NH_3 ranging from $1 \times 10^{-3} \text{ M}$ to $3 \times 10^{-3} \text{ M}$.

Warren (1962) stated that in most biological fluids ammonia exists in two forms, ionized (NH_4^+) and nonionized (NH_3), the relative proportions of which are determined primarily by the pH of the solution. He pointed out that the toxicity depended upon the NH_3 which entered the organism and cell.

Megie et al. (1967) used an aeration method for determining NH_3 . They stated that toxicity of nonionized ammonia (NH_3) was the primary reason for inhibited germination and reduced growth of cotton seedling. Plant growth decreased sharply with increasing NH_3 and levels above 10 ppm were lethal.

Blanchar (1967) developed a direct method to determine partial vapor pressure of NH_3 (P_{NH_3}) in soil air and found that germination of corn seed was inhibited when the initial P_{NH_3} value was 0.156 mm Hg and final values were between 0.077 and 0.104 mm Hg. Allred and Ohlrogge (1964) postulated that free NH_3 associated with DAP fertilizer was toxic to germinating corn. They concluded that NH_3 at a partial vapor pressure as low as 0.125 mm Hg was toxic to corn when it was exposed to this environment for 2 days during the initial stage of germination.

Hunter and Rosenau (1966) measured gaseous NH_3 by a diffusion method. They found germination of corn seeds and growth of seedlings

were greatly inhibited or completely prevented in flasks containing 1 mg or more of gaseous NH_3 . In laboratory experiments, Brage et al. (1960) placed wheat seeds in a petri dish, but not in contact with a mixture of urea and urease. They found that urea to the extent of 20 mg or more produced enough NH_3 gas to prevent any germination of wheat.

Bennett and Adams (1970) considered $\text{NH}_3(\text{aq})$ in the soil solution was the agent of toxicity to plants. They found that symptoms of NH_3 toxicity were evident at $\text{NH}_3(\text{aq})$ concentrations in soil solution in situ above 0.17 mM for sudangrass foliage, and above 0.24 mM for cotton roots. They stated that the critical concentration for incipient NH_3 toxicity was concluded to be 0.15 to 0.20 mM $\text{NH}_3(\text{aq})$.

Colliver and Welch (1970) used a steam distillation method for determining ammonium $(\text{NH}_3 + \text{NH}_4^+)$ -N from anhydrous ammonia. They reported concentrations in excess of approximately 1000 ppm of $(\text{NH}_3 + \text{NH}_4^+)$ -N resulted in significant corn stand reduction. Germination and early growth of corn was retarded when the concentration reached 994 ppm and essentially inhibited when it reached 1628 ppm. By using a similar technique of ammonia determination, Openshaw and Frederick (1970) found that germination of corn and cotton seeds was greater than 87% when anhydrous ammonia added was less than 7 meg/100g soil.

Low and Piper (1961) concluded that NH_3 formed during ammonification caused phytotoxic effects to germinating wheat seeds from as little as 1.3 pounds of biuret per acre applied to urea. Under laboratory studies, Khan and Mandal (1968) observed no emergence of jute seedlings when urea was applied at the rate of 180 and 360 lb-N per acre.

Inorganic sources of N such as anhydrous ammonia or ammonium salts have been reported injurious to plants due to improper placement of fertilizers (Brage et al., 1960; Olson and Drier, 1956). Parr and Papendick (1966) reported that when anhydrous ammonia was injected into soil according to different application schedules, corn yield was considerably reduced at the higher N-levels when compared to equivalent applications of urea and NH_4NO_3 . Yield reduction in this case, they stated, was attributed in part to root damage due to NH_3 toxicity. Colliver and Welch (1970) concluded that such injury generally increased as the rate of anhydrous ammonia application increased.

Brage et al. (1960) stated that enzymatic hydrolysis of urea produced enough gaseous NH_3 to be toxic to germinating seeds, where the seeds were placed near to the mixture of urea or urease in a closed system. Guttay (1957) conducted a series of greenhouse experiments in which he showed that complete fertilizer, applied at the rate of 100 pounds of N (NH_4^+), P_2O_5 , and K_2O per acre in contact with wheat seeds, seriously delayed and curtailed germination and emergence.

Lawton and Davis (1960) reported that contact placement of wheat seeds with 5-20-20 fertilizer ($\text{NH}_4\text{-N}$) at 500 pounds of material per acre delayed and reduced emergence of seedlings and subsequent growth. Applying this mixed fertilizer in a band below, or 1 1/2 inches to the side and 1 1/2 inches below, the seed was not desirable from the standpoint of emergence and growth. Cook et al. (1958) found that the application of 12-12-12 fertilizer per acre with the seeds influenced the emergence of wheat seeds, and at the end of 9 weeks, they observed a 20% reduction in stand. The yields were reduced where the greatest emergence injury occurred. Tillering and other yield components were influenced.

Andrews et al. (1956) observed seedling injury when anhydrous ammonia was applied in contact with germinating seeds. In studying the injurious effects of preplant anhydrous ammonia to germination and early growth of corn, Colliver and Welch (1970) reported severe damage when anhydrous ammonia was applied at 10 cm deep immediately before planting at 5 cm deep. Lorenz et al. (1955) concluded that aqua ammonia placed in the bed under the potato row resulted in low yields and caused severe plant toxicity. They suggested that the toxicity could be lessened by placing the fertilizer farther away from the plant, using split application or delaying application until the crop was well established.

Comparing different methods of fertilizer placements, Stephen and Waid (1963a) reported greater adverse effects of urea when placed near the seeds than when mixed throughout the soil.

Guttay (1957) reported that fertilizer placement in contact with wheat seeds had greater effects on delaying and reducing emergence under dry than moist conditions. Dubetz et al. (1959) proposed that moisture levels alone had no significant effect on the germination of any crop. However, moisture levels in combination with nitrogen fertilizers, they explained, reduced germination, and the reduction became progressively pronounced with decreasing moisture.

MATERIALS AND METHODS

Influence of NH_3 from ammonium phosphate fertilizers on germination, seedling growth, and production of wheat (*Triticum aestivum* L.) were investigated in (I) laboratory, (II) growth chamber, (III) greenhouse, and (IV) field experiments. Specific objectives and experimental procedures are illustrated under each section. The statistical plan is also included for some experiments. The series of experiments are as follows:

I. Laboratory Experiment

Objectives. Although general concepts of volatilization losses and toxicity of ammoniacal-N fertilizers have been recognized and well documented by previous investigators, physio-chemical processes and quantitative determination pertaining to fertilizer-soil-plant systems have not been considered simultaneously and adequately explained. In order to draw a basic sound inference, this laboratory experiment was comprised of 2 investigative studies. The first study is referred to as the " NH_3 Production Study". The investigations involved mainly, i) the patterns of NH_3 release, and ii) the amounts of NH_3 produced from monoammonium phosphate (MAP:11-48-0), diammonium phosphate (DAP:18-46-0) ammonium polyphosphate (APP:15-62-0), and urea ammonium phosphate (UAP:24-42-0) upon reaction with soils varying in moisture and % CaCO_3 contents. The second study is called the " NH_3 Absorption Study". The purposes of this study were: i) develop a procedure of measuring free

NH_3 concentration in soils and define a concentration of NH_3 required to be toxic to spring wheat (*Triticum aestivum* L., 'Fortuna'); ii) evaluate the most susceptible stage of wheat plant development to NH_3 toxicity.

Experimental Procedures

NH_3 Production Study. A "Diffusion Can" with soil and fertilizer mixed, as illustrated in Fig. 1, was designed for this study. The can is a Buckeye, style No. 201, Seamless Tin, outside dimension-- diameter, 7 cm, - depth 5 cm, and total capacity 110 cm^3 . The units consist of two portions, the inner and the outer chambers. The inner cell is a replaceable plastic vial of boric acid solution and located at the center of the diffusion can. The outer is a chamber of soil and fertilizer mixed. The can is kept closed with a lid to allow diffusion to proceed within a closed system and at room temperature. Therefore, the "Diffusion Can" resembles the microdiffusion unit (Bremner and Shaw, 1955) with some modifications.

To evaluate NH_3 production from ammonium phosphate fertilizers on different soils, diffusion cans were set up as a series of laboratory experiments. Six soils at a total of 10 different contents of CaCO_3 were used in "Diffusion Can" experiments. The soil series, pH, and % CaCO_3 equivalent data are in Table 1. There are four ammonium phosphate fertilizers: MAP, DAP, APP, UAP; and three moisture levels: 10%,

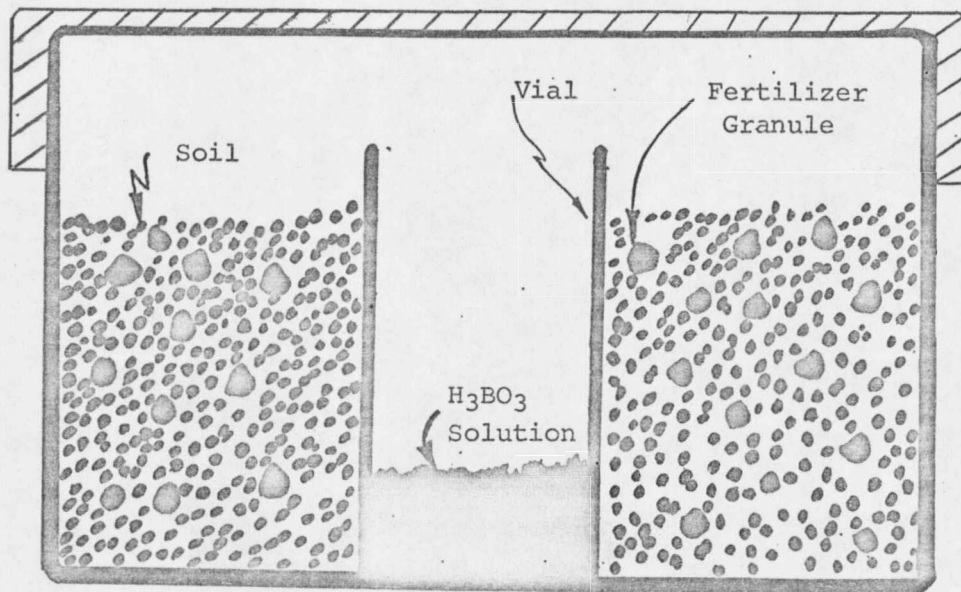


Figure 1. "Diffusion Can" for absorbing NH_3 produced by ammonium phosphate fertilizers reacting with soils.

Table 1. Some Characteristics of Soil used in the Experiments.

Soil No.	Description		Particle size			Initial pH (1:1,H ₂ O)	CaCO ₃ ¹ Equiv. %
	series	1968 Classification Great Group	Dist., % sand silt clay				
1	Bainville cl	Torriorthent	41.2	24.0	34.8	8.0	12.90
2	Wheeler sil	Torriorthent	40.0	50.0	10.0	8.0	12.55
3	Wheeler sil	Torriorthent	40.5	49.2	10.3	7.9	10.05
4	Amsterdam sil	Cryoboroll	40.0	55.0	5.0	7.9	9.20
5	Amsterdam sil	Cryoboroll	38.0	52.0	10.0	7.5	5.50
6	Bainville cl	Torriorthent	35.2	34.0	30.8	7.6	5.00
7	Danvers cl	Argiboroll	30.2	38.9	30.9	7.5	3.20
8	Judith cl	Calciboroll	32.7	43.3	34.0	7.6	3.04
9	Post cl	Natriboroll	12.0	65.8	22.2	7.2	0
10	Amsterdam sil	Cryoboroll	32.5	49.2	17.0	6.8	0

15%, 20%. Therefore, each experiment was set up as a factorial experiment with 12 treatment combinations and replicated 2 times.

In preparing soil and fertilizer mixture for a certain % CaCO₃ content soil, the following techniques were used: one hundred grams of air-dry soil, sieved through 2 mm, was adjusted to specific moisture levels. The moist soil was then thoroughly mixed with ammonium phosphate fertilizer at the rate of 216 mg of N per 100 g soil. A vial of standard 2% H₃BO₃ solution was inserted in each can. The lid was closed and diffusion was allowed to proceed at room temperature for

¹CaCO₃ equivalent, Method for soil characterization, method 23C, USDA Salinity Laboratory Staff (1954).

24 hours before each measurement of NH_3 . Each day a vial of H_3BO_3 solution was replaced for measuring NH_3 by titration against standard 0.005 N H_2SO_4 . The amount of NH_3 was expressed as μg per 100 g air-dry soil. The technique was patterned from the microdiffusion procedure as described by Bremner and Shaw (1955) with some modifications. Each experiment was terminated at the end of 6 days.

NH_3 Absorption study. A preliminary experiment was set up in the laboratory to develop a procedure of measuring free NH_3 concentration. The apparatus consisted of four 6-liter desiccators. Each of the desiccators contained 500 g of air-dry Amsterdam sil soil, 9.2% CaCO_3 , which has adjusted to 20% water content. DAP fertilizer at the rate equivalent to 108 mg of N per 100 g soil was applied into two desiccators. Fifty spring wheat seeds were added into two desiccators in which one was a fertilized-desiccator. After the addition of wheat seed and fertilizer, they were thoroughly mixed with the soil. A beaker containing 10 ml standard 2% H_3BO_3 solution was placed on the racks above seed-fertilizer-soil mixed in each of desiccators. The desiccators were closed. Each day for the period of 6 days, a beaker of H_3BO_3 solution was replaced for measuring NH_3 concentration. The procedure for NH_3 determination was essentially the same as described in " NH_3 Production Study" section. The NH_3 sorption by seeds or seedlings, therefore, was computed by the differences between with-seeds or without-seeds of the two comparable desiccators.

In one NH_3 absorption experiment, a series of "Soil Can" was set up to measure a concentration of NH_3 required to be toxic to wheat seeds or seedlings. The "Soil Can", typical of Buckeye-Seamless Tin, was used. DAP fertilizer was used at the rates of none, 56, 112, and 224 mg of N per 100 g soil. The soil was adjusted to moisture levels of 15%, 20%, 25% and 30% water contents. Ten spring wheat seeds were added to each soil can. The design of experiment was a factorial arrangement and replicated 2 times. The preparation of seed-fertilizer-soil mixed followed general mixing processes as described in previous experiments. The cans were kept closed with lids throughout the experimental period.

After 6 days, the experiment "Soil Can" was terminated, seeds and seedlings were checked to evaluate the effect of NH_3 on germination and seedling growth. The lengths of radicles and coleoptiles were measured individually; roots were counted; and all of plant samples were weighed and recorded.

The determination of NH_3 in seeds or seedlings was made by using a "Distillation Technique" which was technically designed for NH_3 absorption experiments. The apparatus, as illustrated in Fig. 2, is a closed system of a 250 ml Erlenmeyer flask with the top portion connected by glass tube to a vial of H_3BO_3 solution.

Plant samples were washed in 1% H_2SO_4 solution, dried by putting against paper towel, then weighed. The samples were placed in the

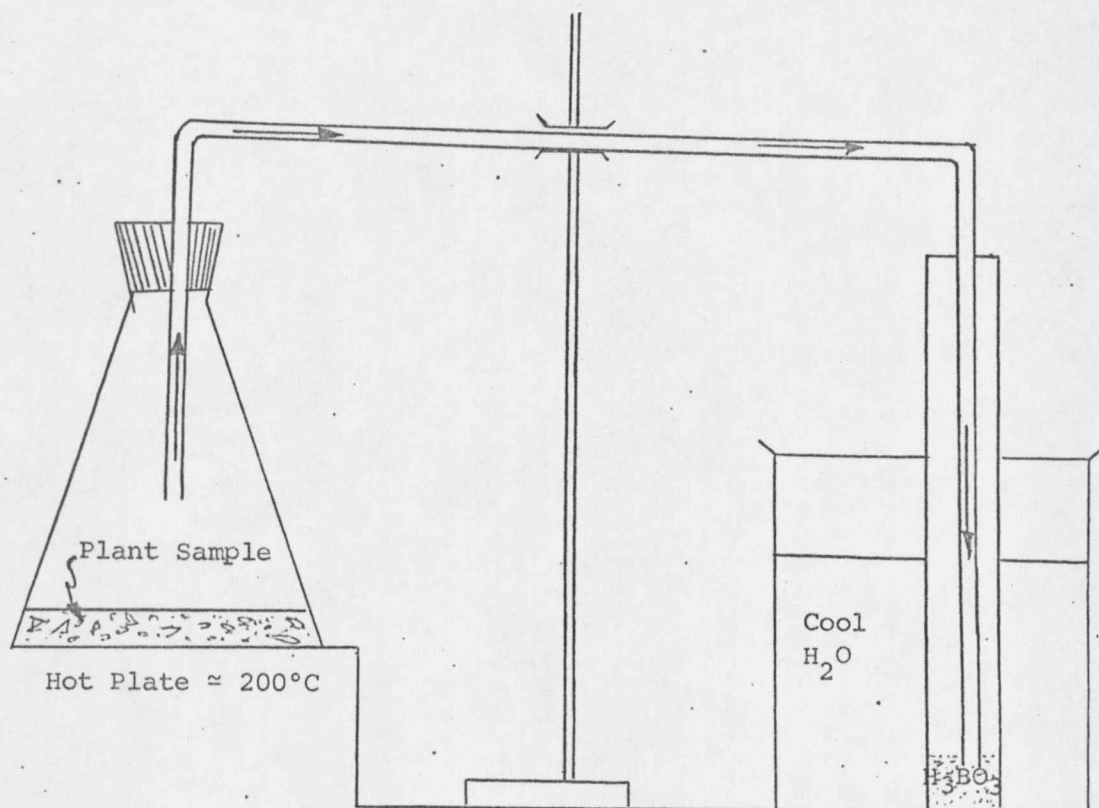


Figure 2. Distillation apparatus for collecting NH_3 gas evolved when fresh plant material is heated on a hotplate.

Erlenmeyer flask in the closed system and were heated over a hot plate at a temperature of about 200°C for 5 minutes. The gaseous NH_3 given off was collected in 2% H_3BO_3 standard solution. Ammonia determination proceeded as described previously. The concentration of NH_3 was expressed as ppm- NH_3 per unit of plant fresh weight.

Another pair of NH_3 absorption experiments were conducted in the laboratory to study the magnitude of NH_3 accumulation over time. In the first experiment, general procedures followed the "Diffusion Can" technique with soil and fertilizer mixed. The design of experiment and treatment combinations was essentially the same as the NH_3 production experiment. One set of treatments had soil and fertilizer mixed without seed. This set was comparable to another which included soil, fertilizer and seed mixed. The soils were Amsterdam sil, 0% CaCO_3 and Wheeler sil, 10.05% CaCO_3 . The NH_3 absorption by seed or seedling was measured by the method as described in a preliminary experiment.

The second experiment of " NH_3 absorption study" was designed primarily to evaluate the most susceptible stage of wheat plants to NH_3 toxicity. Ammonia accumulation over time in seeds or seedlings is the key information. In this experiment the "Soil Can" technique experiment was employed. However, some deviations were made from the previous "Soil Can" so that the terminal times were 1, 2, 3, 4, 5, and 6 days after each experiment was set up. The statistical plan and treatment

combinations of this experiment were essentially the same as the previous "Soil Can" experiment. NH_3 determinations were using "Distillation Technique" as previously described.

II. Growth Chamber Experiments

Objectives. Results from field experiments described in part in annual reports (Smith et al., 1969, 1970, 1971)¹ indicated that there is a relationship existing between NH_3 production and seed germination and/or seedling growth injury of cereal plants. It also appeared that the NH_3 produced from ammonium phosphate fertilizers was associated with lime (CaCO_3) content of soils. Therefore, in order to evaluate whether these relationships exist under controlled conditions, two growth chamber experiments were conducted.

Since the first evidence pointed toward NH_3 production and plant damage, one growth chamber experiment was set up to investigate this observation. In this experiment, several factors which are related to plant damage were investigated by the use of multiple regression analysis as explained below. Five factors were considered simultaneously to provide general applicable parameters to define the deleterious effects of ammonium phosphate fertilizers on germination and seedling growth of wheat.

¹Smith, C. M., E. O. Skogley, and C. Pairintra. 1969. 1970. 1971. Farm test demonstrations. Ann. Reports to TVA (Unpublished).

The design was a central composite rotatable design as described by Cochran and Cox (1957) and modified by Lund.¹ It is a complicated design, but it reduced experimental size and provided all information that was needed. Variables, treatment combinations, and statistical plans are illustrated in Table 2.

A description of the central composite rotatable design with details in Table 2 is as follows:

1. Factors:

Soils (S)	2
Fertilizers (F)	4
Rates (R)	5
Moistures (M)	3
Times ² (T)	5

2. Basic design:

a) It was patterned after the central composite rotatable design of pages 346-7 in Cochran and Cox (1957) for 3 factors in selecting combinations of levels for R, M, T, but:

- i) No star points for M since there were only 3 levels

¹Dr. R. E. Lund, Assistant Professor of Math., Montana State University (Personal communication).

²Time was a variable only as to the days that reactions were allowed to proceed before measurements were made. The response was measured at each designated time.

Table 2. List of variables, treatment combinations, and statistical procedure for growth chamber experiment.

Central Rotatable Design						Factor: Soil ¹	
Trt	Soil	Fert.	Rate	Moist.	Time	Levels	
comb	S	F	R	M	T		
1	1	1	2	1	2	- S ₁ = 0% CaCO ₃ equiv.	
2	1	1	4	1	2	- S ₂ = 5.50 "	
3	1	1	2	3	2	- S ₃ = 9.20 "	
4	1	1	4	3	2	- S ₄ = 10.05 "	
5	1	1	2	1	4		
6	1	1	4	1	4		
7	1	1	2	3	4		
8	1	1	4	3	4		
9	1	1	1	2	3		
10	1	1	5	2	3		
11	1	1	3	2	1		
12	1	1	3	2	5		
13	1	1	3	2	3		
14	1	1	3	2	3		

2³ part
(the eight points constitute a 2³ factorial)

Star points
(the four points are the extra points, the figure formed by these points is called a star)

Central points
(the two points are added at the center)

Factor: Fert.

- F₁ = MAP

- F₂ = DAP

- F₃ = APP

- F₄ = UAP

Factor: Rate

- R₁ = 5.4 mg-N/pot

- R₂ = 54.0 "

- R₃ = 108.0 "

- R₄ = 162.0 "

- R₅ = 216.0 "

Factor: Moist.

- M₁ = 15% soil H₂O

- M₂ = 20% "

- M₃ = 25% "

Factor: Time²

- T₁ = 3 days

- T₂ = 6 "

- T₃ = 9 "

- T₄ = 12 "

- T₅ = 15 "

Repeat above entire set of 14 treatment combination of S and F (S X F = 8 combination levels)

¹Used only 2 soils at one time in the growth chamber because of space limitations.

²Time was a variable only as to the days reactions were allowed to proceed before measurements were made.

- ii) Locate star points at distance $\alpha = 2$ for both R & M. This made design be not really rotatable--variance of \hat{y} was not equal at equal distance from center.
- iii) Use 4 star points and 2 central points. This increased variance of \hat{y} near the center. That is variance of second order terms was increased.
- iv) This required 14 treatment combinations.
- b) For the complete design, each treatment combination of Fertilizers and Soils ($F \times S = 8$) was used with the 14 above treatment combinations. Therefore, the whole experiment required $8 \times 14 = 112$ treatment combinations.
- c) The treatment combinations were set in the growth chamber as a completely randomized design.

3. Estimation and analysis of variance:

<u>Source</u>	<u>D.F.</u>
S	1
F	3
S x F	3
<u>From composite:</u>	
Linear terms on R, M, T	3
Quadratic terms on R, M, T	3
(R & T were estimated well, but M was confounded at some higher order interactions)	
Linear x Linear terms on R, M, T	3
Lack of fit for R, M, T	3

<u>Source</u>	<u>D.F.</u>
<u>Interactions between S X F & Composite:</u>	
Pure error based on central points	8
Error (above mains altogether)	84
Total	111

In addition to the first growth chamber experiment, the second experiment was conducted with more emphasis on the growth of wheat seedlings on calcareous and noncalcareous soils. The design was a factorial experiment where the number of factors were remained the same as the first growth chamber experiment. The only change is the level of some factors which were reduced appropriately to the information that is needed.

Experimental Procedures

In the first growth chamber experiment, factors and levels listed in Table 2 were used and the experiment was repeated 3 times. The control treatments were introduced into the design purposely to describe the original condition as well as to explain the effectiveness of treatment combinations. In general, the experimental procedures were as follows:

1. Growth Chamber. A growth chamber which has two shelves and an internal volume of 154 m^3 was used. It was thermostatically and humidifically controlled at 59°C and 57% relative humidity,

respectively.¹ Twelve fluorescent lights were used as the light sources with alternating periods of 16 hours of light and 8 hours of dark maintained during the course of experiment. Four 10-inch fans were in operation at all times to insure air circulation and to keep uniform temperature and humidity.

2. Soil. Amsterdam sil, 5.5% and 9.2% CaCO_3 contents were used. The soils were taken from the surface approximately 6 inches. They were air-dry then sieved through a 10-mesh screen. A total of 350 g of air-dry soil (4.0% and 2.5% H_2O for the 5.5% and 9.2% CaCO_3 soils, respectively) was used for each pot. Characteristics of soil were shown previously in Table 1.

3. Container. Plastic pots--diameter 11.5 cm and 7.5 cm deep were used.

4. Perlite. A preliminary experiment was conducted to study the evaporation rate in growth chamber by using perlite to cover the top of the soil surface in the experimental pots. Results from several depths of perlite covering indicated that evapotranspiration was reduced to minimum without affecting growth when the pot was covered with perlite from the top to the depth of 1.5 cm.

¹The control of temperature and humidity at 59° C and 57% R.H. was believed to be optimum and appropriate for this study, based on opinions of others experienced in growth chamber use.

5. Soil moisture. The soil was adjusted to desired moisture levels by the following three procedures. First, a total of 350g of air-dry soil, that was required for each pot, was transferred to a flat tray. Each tray of soil was placed over the scale, then the water was added by means of spraying. The adding of water was continued until obtaining a desired total weight. The tray then was removed from the scale and the soil sample was transferred to a piece of paper. The mixing was performed by rolling alternately the ends of the paper. The sample was transferred to the pot corresponding to the moisture treatment specifications.

Second, the processes of adjusting soil water to desired moisture levels were performed essentially the same as previously described, except the mixing. This time the soil and water were mixed by hand. It was observed that both of the two processes introduced some variation into the experiments due to being unsuccessful in getting uniformity and in preventing soil puddling.

In the third repetition of this experiment, the soil was adjusted to desired moisture levels by overnight soaking with water, then hand mixing for each pot before the experiment was performed. This procedure proved to be the best.

6. Seed. Fifteen spring wheat seeds, Fortuna Variety, were used for each pot.

7. Pot Experiment. A diagram of wheat seeds and fertilizer banded in a pot is illustrated in Fig. 3. The preparation of each pot experiment was as follows: About 200 g of moist soil were placed into each pot, then leveled to the mark about 4 cm to the top. Fertilizers were applied corresponding to treatment specifications. It was banded with wheat seeds and in the same plane. The pot was filled with the remainder of the required moist soil. The top portion of the pot was covered with perlite to the mark or approximately 1.5 cm deep. The pots were transferred to a growth chamber.

8. Observation and measurement. At 3-day intervals, certain treatment combinations were removed from the growth chamber for observation and measurement according to Time (T) specifications. Germination was checked as normal, abnormal, and dead. The normal germination is when the length of radicle or coleoptile is greater than 1 cm. If the seedling had either radicle or coleoptile, but not both, it was considered as abnormal whereas the dead was the seed that failed to germinate.

After recording germination, plant samples were washed by running tap water. The height of each plant was measured individually. The length of the roots, which included primary and secondary roots, was measured by averaging of the total roots of each plant. This was done because of the difficulties of measuring root by root of the fibrous roots system of a wheat plant.

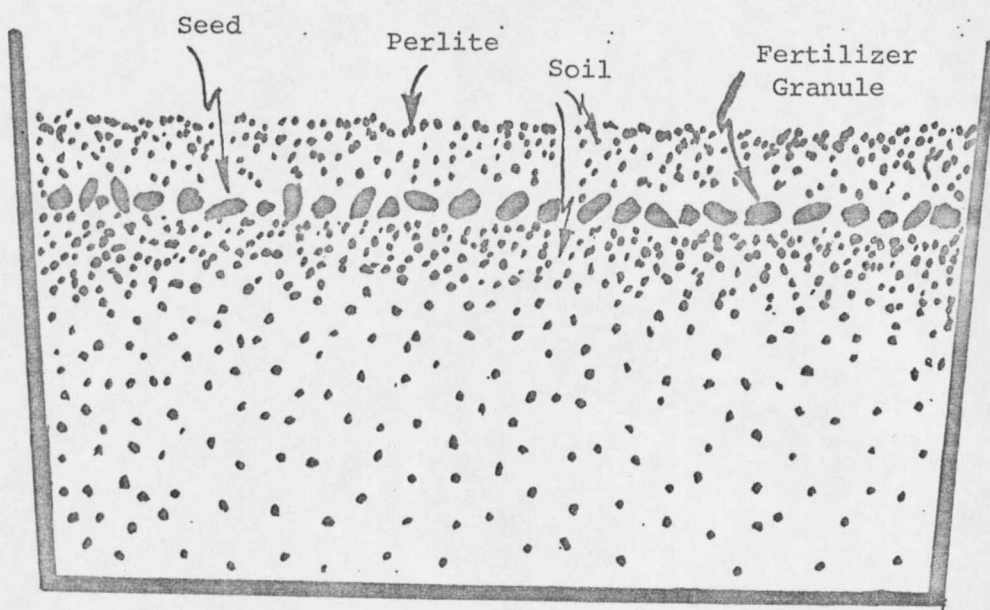


Figure 3. Plastic pots used in growth chamber and greenhouse experiments. Fertilizer and seed were banded on one plane and covered with soil. Perlite was applied on top to reduce evaporation of soil water.

An attempt was made to count the number of primary and secondary roots especially up to the third sampling date or 9-day-old plants. The identification of primary and secondary root systems was according to procedure as illustrated by Boatwright and Ferguson (1967) and Brown (1969).¹ After these measurements were completed, plant samples were weighed and processed for NH_3 determination by the "Distillation Technique", as has been previously described in the laboratory experiment under the " NH_3 Absorption" section.

In the second growth chamber experiment, Amsterdam sil, 0% CaCO_3 and Wheeler sil, 10.05% CaCO_3 contents were used. Soil characteristics are shown in Table 1.

The experimental procedures were essentially the same as the first growth chamber experiment but with a different design. This was a factorial experiment with the factors and levels as follows with additional details in Table 2.

<u>Factor</u>	<u>Level</u>
1. Soil (S)	2 (S_1 and S_4)
2. Fert. (F)	4 (MAP, DAP, APP, UAP)
3. Rate (R)	2 (R_2 and R_5)
4. Moist. (M)	2 (M_1 and M_3)
5. Time (T)	2 (T_2 and T_4)

¹Brown, P. L. 1969. Wheat and barley-germination, morphology and stages of developments. Barley and wheat workshop. Mont. State Univ., Bozeman, June 29-July 2, 1969.

III. Greenhouse Experiments

Objectives. Two experiments were conducted in the greenhouse.

The first experiment was set up to study some characteristics of wheat seedlings after they were subjected to different concentrations of NH_3 . The main purpose of this experiment was to investigate the toxic effect of the NH_3 that entered the seed through the seed coat, and the seedling through the radicle or roots. This experiment is referred to as " NH_3 Toxicity Studies".

The second experiment was designed to evaluate the growth of spring wheat seedling in early stage as affected by ammonium phosphate fertilizers banded with seeds in pot experiments. The effects of an additional nutrient solution on growth of damaged plants were also evaluated. The main objective of this experiment was to compare the recovery of injured plants for different soils and fertilizers. Therefore, it is referred to as "Growth Study".

Experimental procedures

NH_3 Toxicity Studies. Part of this experiment was performed in the laboratory as follows: three hundred spring wheat seeds were soaked in distilled water, then they were separated into two groups. The first group was placed on a rack over the NH_4OH solutions in the dessicators. The solution concentrations were: none, 0.25, 0.50, 0.75, and 1.00 ppm-N. After the seeds were subjected to partial vapor

pressure of NH_3 , they were planted in pots. The general procedures were essentially the same as the first group. Therefore, the two sets of pots were arranged in a randomized complete block design with 2 replications. Plants were watered daily, and the experiments were carried out for 15 days. At the end of the experiment, fresh weight, dry weight, and plant height were recorded.

Growth Study. A factorial design was employed for the "Growth Study". A list of variables, treatment combinations, and statistical plan is illustrated in Table 3. The experimental procedures were as follows:

1. Time. The experiment was conducted for a total period of 49 days. They were in growth chamber (as illustrated in Growth Chamber Experiment) for 9 days, then were transferred to grow in greenhouse for 40 days.

2. Soil. Two soils were used. Soil samples were prepared and the required amounts were essentially the same as previously described in Growth Chamber Experiment. Soil characteristics are shown in Table 1.

3. Pot. It was a plastic pot--diameter 11.5 cm and 14.5 cm deep, the same as described before.

4. Perlite. As a result of preliminary trial, it was decided to place perlite into two portions of each pot, the bottom and the top.

Table 3. List of variables, treatment combinations, and statistical plan for "Growth Study" in the greenhouse.

Treatment combination	Soil S	Fert. F	Rate R	Moisture M	Nutrient Solution N
1	1	1	2	1	1
2	1	1	2	1	2
3	1	1	2	3	1
4	1	1	2	3	2
5	1	1	5	1	1
6	1	1	5	1	2
7	1	1	5	3	1
8	1	1	5	3	2

Repeat 8 treatment combinations
for Soil (S_1 and S_4) and Fert.
(2 x 4).

Factor = Soil

S_1 = 0% CaCO_3
 S_4 = 10.05% CaCO_3

Factor = Fert.

F_1 = MAP
 F_2 = DAP
 F_3 = APP
 F_4 = UAP

Factor = Rate

R_2 = 54 mg-N/pot
 R_5 = 216 "

Factor = Moist.¹

M_1 = .15% soil H_2O
 M_3 = 25% "

Factor = Nutrient solution

N_1 = 54 mg-N/pot as KNO_3
 N_2 = 216 " "

¹ Soil moisture differences were partially maintained during greenhouse period.

At the bottom of each pot the wet perlite was filled to about 7.5 cm thick. It was used to prevent compaction of the soil and for extra area for root growth.

5. Soil moisture. The soils were adjusted to desired moisture levels by overnight soaking with water in the tub, then hand mixing. The required amount of moist soil was transferred to each pot to the exact weight.

6. General procedures. Wheat seeds-fertilizer banded in pot followed the method described in Growth Chamber Experiment.

After 9 days, pots were transferred to the greenhouse. Water was uniformly added or added when needed, but strictly to treatment specifications. Nutrient solutions of KNO_3 were prepared according to the method described by Skogley (1969)¹ and were added corresponding to treatment specifications. The nutrient solutions were added every 6 days, totally 1000 ml for each rate.

After 49 days, plants were harvested. Plant parts, tops and roots were separated then oven-dried at 65° C for 2 days. The plant dry weight was recorded.

¹Skogley, E. O. 1969. Nutrient solution. (Personal communication).

III. Field Experiments

Research on the field aspects of ammonium phosphate injury to germination and growth of wheat was started in the fall of 1967 by Smith.¹ These experiments and later ones on barley and wheat were designed to evaluate the differential influence of ammonium phosphate fertilizers on germination, seedling injury and ammonia production. The main purpose was to define the problems associated with losses of stand from use of DAP under certain field conditions and to compare results with other ammonium phosphate fertilizers. Farmer experiences had shown that losses of stand occurred from use of DAP, and the conditions to cause these losses were not always clear.

This dissertation emphasizes the influences of ammonium phosphate fertilizers on germination, plant growth, and ammonia production, as related to different kinds and rates of fertilizer and CaCO_3 and moisture contents of soils.

Field experiments employed the use of a deep furrow drill which banded the fertilizer with the grain seed. Soil samples were taken at different dates after planting. These were handled by special techniques as explained later. Results of field experiments established that differences in plant growth effects and in NH_3 measured in soil

¹Smith, C. M. 1968. Farm test demonstrations. Ann. Report to TVA (Unpublished).

samples were associated with ammonium phosphate sources and CaCO_3 in soils.^{1,2}

The field experiments reported in this thesis were conducted in the crop year 1970-1971. They were located in the Gallatin Valley as follows: Irving Snyder farm, Amsterdam sil, eroded phase, 9.2% CaCO_3 ; Don Jones farm, Amsterdam sil, 0% CaCO_3 . The crop was winter wheat, variety Winalta. Soil characteristics are presented in Table 1.

Seeding was September 22 and 27, 1971, on the Jones and Snyder locations, respectively. The drill was a Minneapolis Moline press drill with row spacings of 30 cm. Spreaders were used at the bottoms of seed sprouts resulting in a fertilizer-seed pattern about 6.5 cm wide within the row. Winalta winter wheat was seeded at the rate of 66 kg/ha (60 lb/A).

Variables under study were as follows:

1. Sources: MAP (11-48-0), DAP (18-46-0), APP (15-62-0), and UAP (24-42-0).
2. Rates: There were 4 rates based on the N in each fertilizer. P rates were not adjusted to be equal because

¹Smith, C. M. 1968. Farm test demonstrations. Ann. Report to TVA (Unpublished).

²Smith, C. M., et al. 1969, 1970, 1971. Farm test demonstrations. Ann. Reports to TVA (Unpublished).

previous years' data indicated additional detrimental effects of additional P applied in the seed row. Rates were: 11, 22, 33, and 44 kg-N/ha (10, 20, 30, and 40 lb-N/A).

3. Soils: Two locations, or two soils, including Amsterdam si. 1. at 9.2% CaCO_3 and 0% CaCO_3 contents.

Field soil and plant samples studies. Soil samples were taken from experimental plots at depth of seed and fertilizer. Four soil core samples were obtained from each fertilizer treatment of two field replications using a soil sampling tube 3.5 cm in diameter. Samples were from the depth of 2.5 to 6.5 cm. Three cores were taken from each of two rows, cores were mixed and transferred into soil cans. All cans were sealed by masking tape and were immediately quick frozen by dry ice in an insulated styrofoam box. They were kept in a deep freeze until being analyzed. Samples were taken at three day intervals for five sampling dates.

Microdiffusion method similar to Bremner and Shaw (1955) was used for determining NH_3 and the procedures were as follows:

1. The can containing frozen soil was opened and three small cores of soil (5 mm diameter) removed and transferred to the outer chamber of a Conway microdiffusion dish.

2. One ml of 2% standard H_3BO_3 solution from a microbiuret (1/10 ml) was charged into the inner cell of the microdiffusion dish.

3. The disc was immediately sealed and diffusion process allowed to proceed for 24 hours.

4. The H_3BO_3 solution was then transferred by micropipet to 50 ml flask, one ml of distilled water added, and titrated with 0.005 N H_2SO_4 .

On May 10 and 12, 1971, early growth stage plant samples were collected from 2 rows 100 cm long for seedling growth studies. Plants had essentially completed tillering and were in stem elongation, or stage 5 of Feekes (1941) scale. Measurements were as follows:

1. Stand counts
2. Stem counts
3. Primary root counts
4. Secondary root counts
5. Plant height
6. Oven-dried weight of roots
7. Oven-dried weight of top

Plant samples were taken from two replications of all ammonium phosphate treatments plus the check from both locations by carefully digging the lower portion of the roots with a spade fork. With caution

soil was partially shaken off the roots and samples were sealed in plastic bags and kept cool in insulated boxes.

Samples were brought to the laboratory where the soil was washed free from the roots by running tap water. Plant height was made by averaging the majority of leaves when plants were stretched out on the leveling table. Primary and secondary root counts were performed according to specifications as illustrated by Boatwright and Ferguson (1967) and Brown (1969).¹ Crown counts and stem counts were made. After these measurements were completed, tops and roots were separated by clipping just above the point of attachment of the roots. The separated plant samples were put into a paper sack and dried at 110°C for 48 hours, then dry weights were recorded.

¹Brown, P. L. 1969. Wheat and barley-germination, morphology and stages of developments. Barley and wheat workshop. Mont. State Univ., Bozeman, June 29-July 2, 1969.

RESULTS AND DISCUSSION

I. Laboratory Experiments

There were 2 main investigations involving NH_3 in which one was on NH_3 production from 4 ammonium phosphate fertilizers reacting with different soils, and the other on NH_3 absorption by seeds or seedlings of wheat.

NH_3 production. Production of NH_3 by 4 ammonium phosphate fertilizers upon reaction with soils varying in % CaCO_3 and soil moisture contents was evaluated in "Diffusion Can" experiments. The following discussions are about parts of the data presented in summary type tables. Complete data are presented for your reference in Appendix Tables 1 through 10.

Total amount of NH_3 produced from 4 ammonium phosphate fertilizers is in Fig. 4. These are overall means of 360 samples for each of 4 fertilizers reacting 6 days in 10 soils with 3 moisture levels and 2 replications. The soils contained CaCO_3 percentages as follows: 0, 0, 3, 3.2, 5, 5.5, 9.2, 10, 12.6, and 12.9. Relatively, total NH_3 production was in the order of magnitude: $\text{UAP} \gg \gg \text{DAP} \gg \text{MAP} > \text{APP}$ or by the ratio of 18:4.5:1.5:1. This relationship of NH_3 production was generally true for any soil type, but the absolute values varied depending upon % CaCO_3 and moisture in the soil. UAP produced the most NH_3 regardless of levels of CaCO_3 or moisture in the soil.

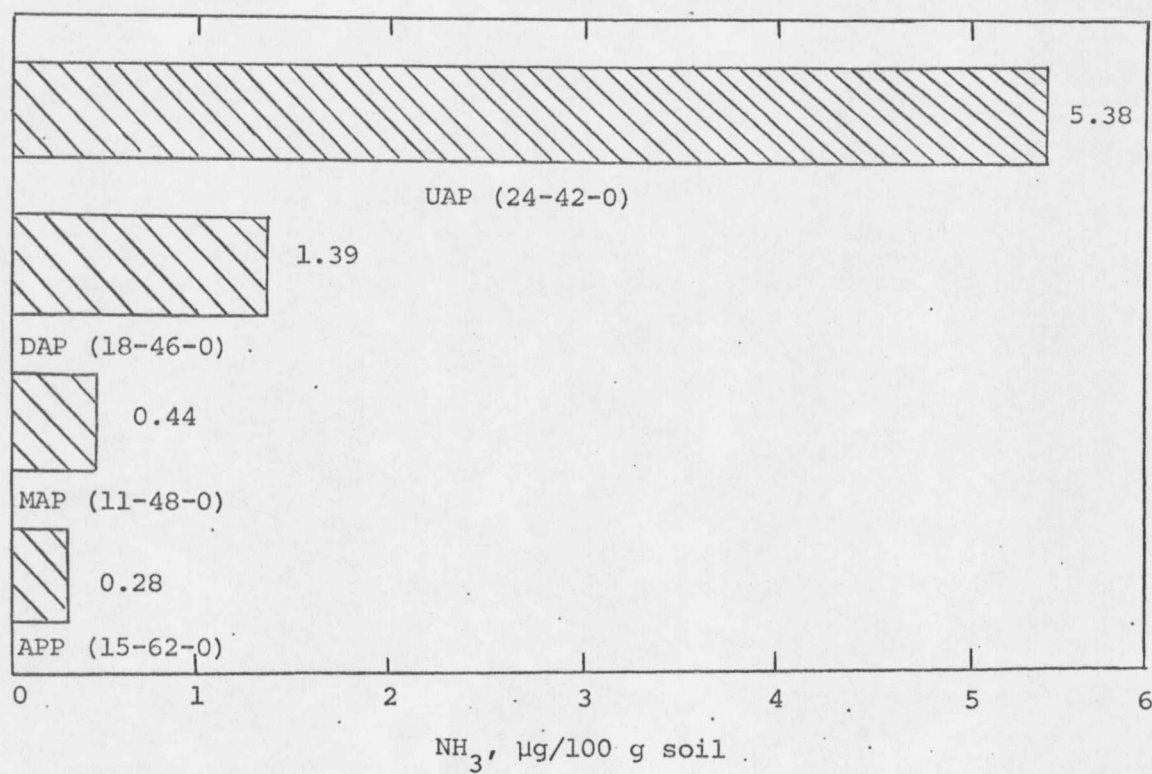


Figure 4. Total NH_3 production for 6 days from MAP, DAP, APP, and UAP fertilizers as determined in a diffusion can. Average of 10 soils, 3 moisture levels, and 2 replications.

The patterns of NH_3 produced from the 4 fertilizers are illustrated in Table 4 in the form of relative frequency distributions, by days, of the maximum amounts of NH_3 . This does not, however, represent total production. The frequency distribution of maximum NH_3 production indicated that for any soil there was no evidence of a maximum NH_3 peak production from UAP in the first day. However, UAP did have a large amount produced on that day.

Table 4. Number of soils with a maximum NH_3 production in each of 6 days. Ten soils averaged over 3 H_2O levels and 2 reps.¹

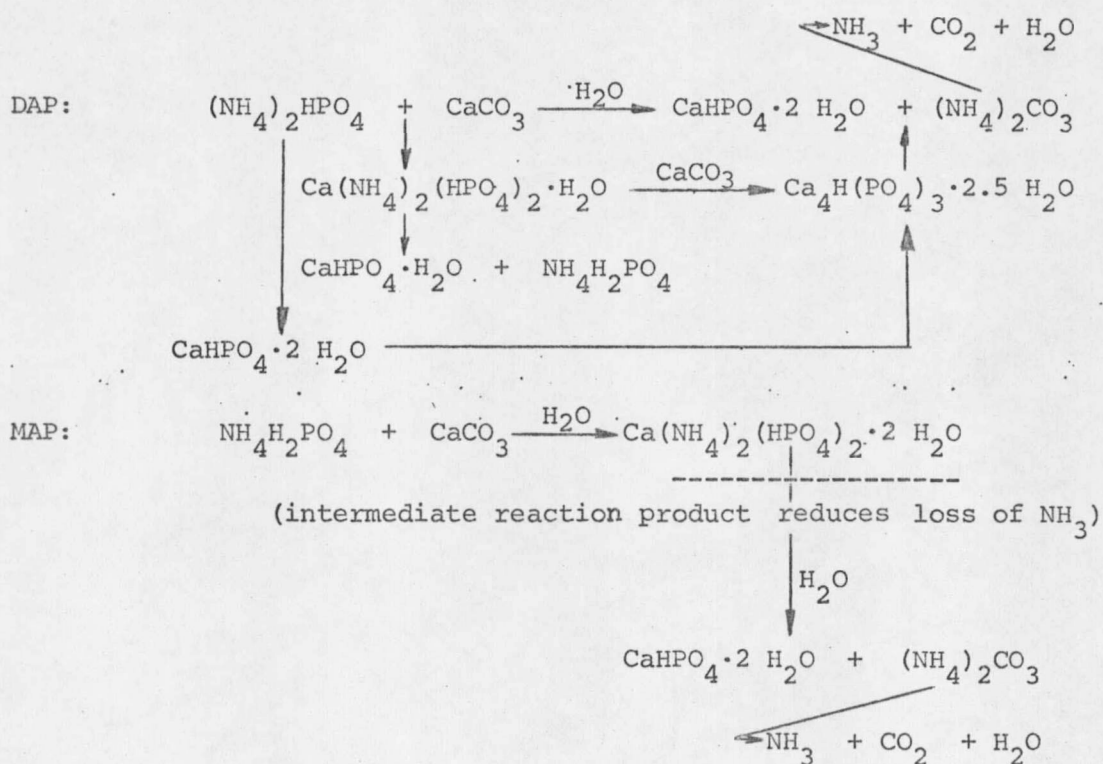
Fert.	No. of soils with maximum NH_3 production in each of 6 days					
	1	2	3	4	5	6
MAP	4	1	4	1	0	0
DAP	4	3	2	1	0	0
APP	4	2	1	3	0	0
UAP	0	2	3	3	1	1

¹Complete data are given in Appendix tables 1 through 10. Soils are described in Table 1. Also, refer to Figures 5 & 6.

At the same time, there was no maximum NH_3 production of MAP, DAP, and APP at the fifth and sixth day periods. These evidences indicate that UAP must have a slower chemical reaction rate than the others after an initial high release. This presents a contradictory result as one could expect regarding chemical reaction rate of UAP under natural conditions and many soil types. The slower rate presumably was due to

limited biological activity needed to accelerate the hydrolysis process under the conditions of this study.

When comparing MAP and DAP fertilizers using the frequency distribution information, DAP reacted faster than MAP in producing maximum amounts of NH_3 in all soils in this study. Terman and Hunt (1964) explained that MAP and DAP have similar reactions in calcareous soils, but due to $\text{Ca}(\text{NH}_4)_2(\text{HPO}_4)_2 \cdot \text{H}_2\text{O}$, the intermediate reaction product of MAP, NH_3 volatilization is largely prevented from reactions of MAP. Using their interpretations and other information from chemistry, the schemes of reactions are presented as follows:



According to the above equations, especially for DAP, the reactions partly depend upon moisture levels of the soils. The results of this experiment have established the same phenomena. The data and discussion are presented later in this section.

Terman and Hunt (1964) explained further that APP also reacts with CaCO_3 in soil and when the initial reaction is completed, the addition of more Ca causes a reaction similar to that of DAP. The formation of $\text{Ca}(\text{NH}_4)_2\text{P}_2\text{O}_7 \cdot \text{H}_2\text{O}$ is the intermediate reaction product that prevents NH_3 loss for this fertilizer material. It should be mentioned that APP, which did produce some gaseous NH_3 , was the best fertilizer for small grain yield on calcareous soils when it was banded with or near the seed.¹ This may be due to the lower amount of NH_3 produced or to some other superiority properties as stated by Phillips and Webb (1972).

Many previous investigators (Gasser, 1964; Meyer et al., 1961; Terman and Hunt, 1964) have explained the losses of NH_3 among N fertilizers in terms of urea hydrolysis or the reaction of certain acid radicals of ammonium salts with calcium compounds in soil. The results obtained from this experiment indicate that NH_3 can be volatilized from UAP upon reacting with soil containing 0% CaCO_3 (see Table 5).

¹Smith, C. M., E. O. Skogley, and C. Pairintra. 1969. 1970. 1971. Farm test demonstrations. Ann. Report to TVA (Unpublished).

Table 5. Ammonia production as affected by 2 soil moisture levels and 4 ammonium phosphate fertilizers reacting on 4 soils of different CaCO_3 percentages.

Fert. & Rate mg-N/ 100g soil	Soil H ₂ O%	Total NH ₃ Production ¹ , 6 days, NH ₃			
		Bainville sil 12.90% CaCO ₃	Wheeler sil 10.05% CaCO ₃	Amsterdam sil 5.50% CaCO ₃	0% CaCO ₃
-----mg/100g soil-----					
<u>MAP (11-48-0)</u>					
216	10	10.34	3.35	1.46	.12
	20	3.84	1.90	.77	.07
<u>DAP (18-46-0)</u>					
216	10	23.31	9.85	6.32	.77
	20	15.48	5.35	5.00	.55
<u>APP (15-62-0)</u>					
216	10	7.13	2.10	1.20	.09
	20	3.57	1.05	.77	.04
<u>UAP (24-42-0)</u>					
216	10	23.28	22.75	17.23	20.17
	20	18.80	16.80	13.10	12.24

¹Total NH_3 for 6 days, average of 2 replication. Complete data for 10 soils are in Appendix tables 1 through 10.

However, the presence of CaCO_3 in soil was a primary factor accentuating the amount and sometimes the rate of NH_3 production. Note the change in NH_3 amounts produced at 20% H_2O from about 1200 $\mu\text{g}/100\text{ g}$ soil from an Amsterdam soil at 0% CaCO_3 to nearly 1900 from a Bainville soil at 12.9% CaCO_3 . Other fertilizers show greater percentage increases with increased CaCO_3 , as is discussed later.

As shown in the LITERATURE REVIEW, many researchers have found that patterns of NH_3 release from various ammonium phosphate fertilizer sources differ and depend upon soil moisture, pH, texture, % CaCO_3 , and other properties of soil.

In the experiments reported in this thesis, the total amounts of NH_3 produced from 4 ammonium phosphate fertilizers were found to be inversely proportional to soil moisture, i.e., the drier the soil; the greater amount of NH_3 was released. Averaging all soils, the total 6-day production was in the soil moisture order of 10% > 15% > 20%. However, the amount of NH_3 produced in the first day of experiment for all soil types was in the order of magnitude: 20% > 15% > 10%. This first-day effect is presumably the direct result of more rapid hydrolysis of the fertilizer with greater moisture.

In the second day after the reaction was started, and for each following day, the drier soil produced a greater amount of NH_3 from each of the 4 ammonium phosphate fertilizers. An example of the effects of soil moisture on NH_3 production for MAP and DAP with 10% and

20% soil H_2O is shown in Fig. 5. Furthermore, the results indicated significant interactions among kinds of fertilizers and soil moisture (F x M) for all soils except Amsterdam sil with 9.2% $CaCO_3$ content, not shown in the above table.

The effect of soil moisture level on NH_3 production from each fertilizer source also varies depending upon % $CaCO_3$ soil. A comparison of effects of moisture for MAP, DAP, APP and UAP is illustrated in the above Table 5 for the following soils: Bainville sil, 12.90% $CaCO_3$; Wheeler sil, 10.05% $CaCO_3$; and Amsterdam sil at 5.50% and 0% $CaCO_3$.

The data suggest that, for all 4 ammonium phosphate fertilizers, the soil H_2O at 10% produced greater amounts of NH_3 than at 20% soil H_2O . Moreover, for each moisture level of the same fertilizer material, the magnitude of NH_3 measured decreased corresponding to the decreasing % $CaCO_3$ content of the soils. The evidence indicates that an SxFxM interaction probably exists.

One of the primary reasons for this research is the study of the influence of % $CaCO_3$ in soil on NH_3 volatilization for 4 ammonium phosphate fertilizers. The total amount of NH_3 produced from 4 ammonium phosphate fertilizers reacting in 10 soils arranged in the order of magnitude % $CaCO_3$ in soil is presented in Table 6.

Some points can be made from observing these data. The magnitude of NH_3 produced from each fertilizer was in the order

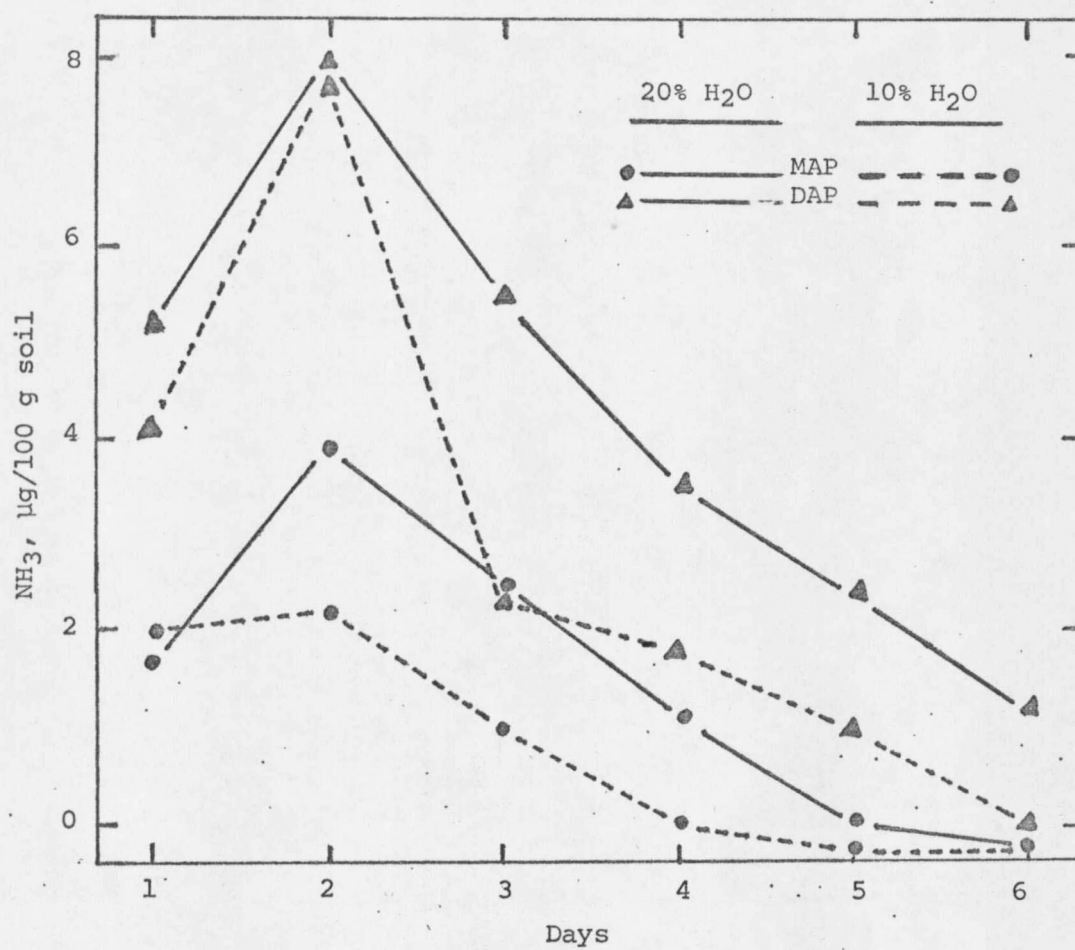


Figure 5. NH_3 production with time as affected by MAP and DAP fertilizers at 10% and 20% soil H_2O . Wheeler sil, 12.55% CaCO_3 .

Table 6. Total NH_3 production for 6 days from 4 ammonium phosphate fertilizers on 10 soils of different % CaCO_3 contents and at 10% soil H_2O .¹

Soil series	CaCO ₃ content	NH ₃ Production from fertilizers			
		MAP	DAP	APP	UAP
-----NH ₃ , g x 10 ⁻⁸ /100g soil-----					
Bainville cl	12.90	1034	2331	713	2328
Wheeler sil	12.55	985	2494	698	5342
Wheeler sil	10.05	335	985	210	2275
Amsterdam sil	9.20	301	804	177	2333
Amsterdam sil	5.50	146	632	120	1723
Bainville sil	5.00	484	852	141	1764
Danvers cl	3.20	65	385	85	1813
Judith cl	3.04	18	246	38	1786
Amsterdam sil	0	12	77	9	2017
Post cl	0	33	812	62	16659

¹All data are in Appendix tables 1 - 10. Soils described in Table 1.

corresponding directly to % CaCO_3 in soil. The only exceptions in these 10 soils were: Either Bainville at 12.9% CaCO_3 or Wheeler at 12.5%; Bainville at 5% CaCO_3 for MAP and DAP; and Post at 0% CaCO_3 for all fertilizers. The most serious discrepancy occurred with the Post soil, especially for DAP and UAP. The same relative data were obtained for all soil moisture levels. No logical explanation of these results was evident from this research. However, Post is a high magnesium soil, which could be a factor. Another possibility might be a reaction

between the fertilizers UAP and DAP and "fixed" or interlayer NH_4^+ in the clay.

There are at least two reasons for the relationship of NH_3 production and CaCO_3 : i) A higher degree of calcium saturation of the soil exchange complex with an increasing amount of CaCO_3 and an associated increase in pH; and consequently, ii) an increased OH^- activity in the soil solution, thus increasing NH_3 production (Wahhab et al., 1957). By using regression analysis, effects of CaCO_3 in soils and NH_3 production can be related. However, several attempts in the computer failed to produce curves that were representative of the data. As a result, eye-fitted curves were drawn and good relationships were shown between % CaCO_3 contents of the soil and NH_3 production.

The slopes of the curves appear to be similar although intercepts are different. The estimated intercepts in $\mu\text{g NH}_3$ per 100 g soil, at zero % CaCO_3 were as follows: MAP = 1.0; DAP = 3.5; APP = 0.5; UAP = 18. The curvilinear nature of each curve indicates no great change in NH_3 production in the relatively flat lower ranges of CaCO_3 contents of soils. Then, as the % CaCO_3 in soils increases, the greater is the NH_3 production. The graph of NH_3 production in Fig. shows that each fertilizer curve ascends with rapidity as % CaCO_3 in the soil increases above a certain amount used in this study. The good example is the NH_3 production from UAP fertilizer on Post cl with 0% CaCO_3 . If the reactions were entirely carbonate dependent, NH_3

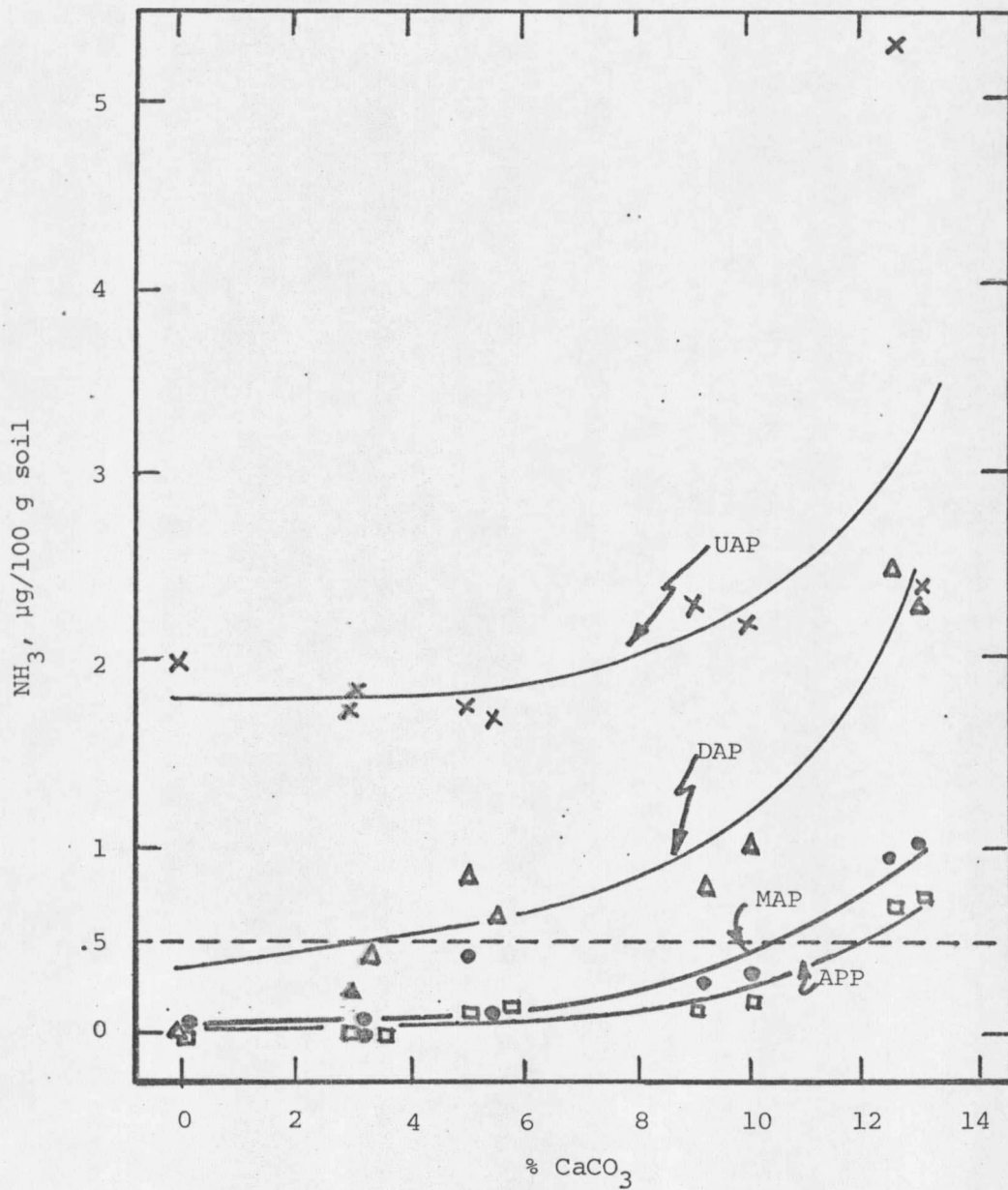


Figure 6. The relationship between NH_3 production using Diffusion Can technique and $\% \text{CaCO}_3$ content of 9 soils. Shown are regression (eye-fitted) lines of MAP, DAP, APP, and UAP fertilizers.

production should have been low but other factors were operating. Also, if carbonate were the only system operating, NH_3 production should approximate a straight line as related to % CaCO_3 .

Using information from the relationship of NH_3 production and % CaCO_3 content of soils, one could predict the effect of 4 ammonium phosphate fertilizers as to the amount of NH_3 produced and its effect on growth of plants due to the toxicity of NH_3 . For example, if $0.5 \mu\text{g}$ $\text{NH}_3/100 \text{ g}$ soil (by this technique) is arbitrarily used as criteria for possible damage to wheat from fertilizer banded with seed, the approximate CaCO_3 levels where such would occur for the 4 ammonium phosphate fertilizers in this study were as follows: MAP 10.5%; DAP 3.5%; APP 12%; UAP, cannot use at all.

An attempt was made to determine the relation of NH_3 production and pH of 10 soils. No satisfactory result was obtained partly because of lack of soils in the acid range. Previous researchers (Du Plessis and Kroontje, 1964; Wahhab et al., 1957) reported that in acid, neutral or alkaline soil, the NH_3 volatilization was governed by pH of the soil system.

Results from this experiment pointed out that % CaCO_3 appears to be a useful system to relate soils with possible NH_3 production. Statements by Larsen and Gunary (1962) and Terman and Hunt (1964), therefore, are in agreement since the results of their experiments pointed out that NH_3 volatilization was a carbonate-dependent system.

However, there are possibilities that other factors may have direct relation with NH_3 production by ammonium phosphate fertilizers.

One of the evaluations in this section is to examine the validity of the "Diffusion Can" technique in measuring NH_3 . By using the stepwise analysis, the data for the means of NH_3 from 4 ammonium phosphate fertilizers for 12.90% and 12.55% CaCO_3 were compared. The method of calculation follows the method of paired-samples as described by Snedecor and Cochran (1967). The data are in Table 7.

Table 7. Mean NH_3 Production from 4 ammonium phosphate fertilizers by using "Diffusion Can" of 2 soils; Bainville cl, 12.90% CaCO_3 and Wheeler sil, 12.55% CaCO_3 .¹

Fertilizer	Bainville cl 12.90% CaCO_3	Wheeler sil 12.55% CaCO_3
	----- NH_3 , $\mu\text{g}/100$ g soil-----	
MAP	1.21	1.29
DAP	2.86	3.52
APP	.87	.87
UAP	3.66	5.96
Mean	2.15	2.91

¹ Average over 3 moisture levels, 2 reps, 6 days.

As a result, t-test value for this data is = 0.8 which is non-significant difference in NH_3 production of two sample means. Therefore, it may be concluded that within the range of 0.35% difference in % CaCO_3 there is no statistical difference between NH_3 production from

2 soils. The results of this test suggested that at 12.55% or 12.90% CaCO_3 content, 2 soils produced the same amount of NH_3 from each of 4 ammonium phosphate fertilizers. Therefore, the method "Diffusion Can" would be a good tool in measuring NH_3 from farmer samples to get an idea of their potential hazard with ammonium type fertilizer.

NH_3 sorption by spring wheat seeds or seedlings. A preliminary experiment was set up to evaluate a possibility of NH_3 sorption by wheat seeds or seedlings from external media. The experimental apparatus consisted of series of closed desiccators containing soil-fertilizer-wheat seed mixed, with H_3BO_3 containers placed on racks above the soil samples. Daily NH_3 determinations were made based on the diffusion method and has been described in the NH_3 absorption study in the MATERIALS AND METHODS section.

For 6-day periods in closed system, wheat seeds and seedlings demonstrated desorption or release of NH_3 from seeds and absorption of free NH_3 gas by seeds and seedlings that was derived from soil and fertilizer. The results are presented in Table 8. It is not understood why there was a greater NH_3 desorption at the first day for the fertilizer system as contrasted with the nonfertilized.

It was assumed that the NH_3 released from wheat seeds during the beginning stage of germination was generated by deamination of the amino acids upon breaking down of seed protein. The NH_3 thus released, according to Webster (1959), is generated according to schemes:

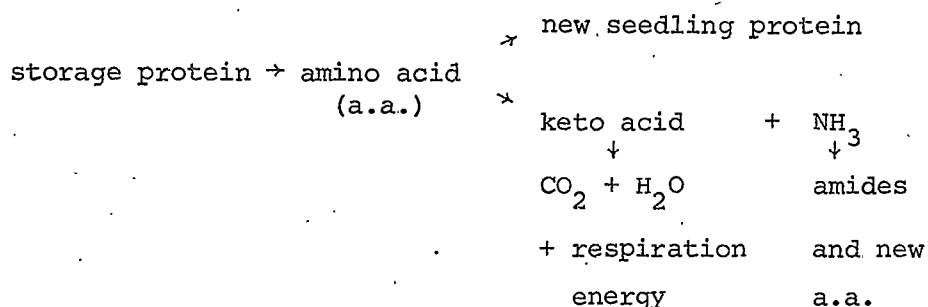


Table 8. Sorption of NH_3 by spring wheat seeds and seedlings. Amsterdam sil, 9.20% CaCO_3 . Preliminary desiccator experiment.

Fert. & Rate	Soil	NH ₃ Sorption/seed or seedling in 6 days					
		1	2	3	4	5	6
DAP (18-46-0)	H ₂ O						
N, mg/100g soil %							
check	20	-.027	-.005	+.016	+.009	+.010	+.004
108	20	-.171	+.315	+.690	+.354	+.135	+.070

- Denotes NH_3 desorption from seeds

+ Denotes NH_3 absorption by seeds and/or by seedlings

At the later stage of germination, it was found that NH_3 was absorbed by wheat seedlings presumably after the emergence of radicles.

The data in Table 8 with a plus sign show large relative differences occurring with time and with fertilizer treatment. The nonfertilized check showed a peak on day 3. This NH_3 must have originated from organic decomposition processes in the soil. The fertilized treatment showed increases from -.171 $\mu\text{g}/100\text{g}$ plant fresh weight the first day to a maximum of .690 $\mu\text{g}/100\text{g}$ the third day. After the third day, apparently

the NH_3 was transformed rather rapidly. What day, or days, that NH_3 absorption reaches a maximum has importance as related to time-release of NH_3 from different fertilizer reactions in different soils.

The process of NH_3 entering into plants cannot be stated with certainty at present. Warren (1962) reasoned that NH_4^+ does not cross a cellular barrier at an appreciable rate, but NH_3 does so, freely. The mode of nonionized ammonia (NH_3) transport throws doubt on the diffusion process where movement of ions from an external medium into the cell is by concentration gradient. It more nearly follows a passive phenomena as proposed by Olsen (1969). When NH_3 is taken up by cells, it is usually incorporated most readily into glutamate, followed by its incorporation into aspartate and alanine (Webster, 1959).

Ammonia sorption with time by spring wheat seeds or seedlings originating from ammonium phosphate fertilizers was measured in diffusion cans. Large differences in NH_3 absorption shown in the preliminary experiments suggested this technique could be used to identify different rates of reactions occurring with fertilizer and soil variables. The concentration of NH_3 that was found in seeds or seedling by using this technique (closed system) would not necessarily account for the total NH_3 produced from fertilizer-soil reactions. One reason was the competition for NH_3 between seeds or seedlings and H_3BO_3

solution. Moreover, the daily changing of vials of H_3BO_3 solution might permit NH_3 gas to escape from the system.

Two soils were used which had large differences in CaCO_3 . Otherwise, these soils were very similar. The Amsterdam sil soil with 0% CaCO_3 and the Wheeler sil with 10.05% CaCO_3 were subjected to seed and fertilizer, and to moisture levels of 15%, 20% and 25%. Measured NH_3 was recorded and subtracted from the NH_3 produced in the same system, but without the seeds.

Data in Table 9 illustrate the magnitude of NH_3 production from fertilizer sources in systems with and without seeds and NH_3 sorption by spring wheat seeds or plants for the 10.05% CaCO_3 soil. The patterns of NH_3 absorption by seeds or seedlings were closely related with NH_3 production, i.e., the more NH_3 produced, the more NH_3 was absorbed. For all fertilizers, the maximum absorption of NH_3 occurred at the third day for 10% CaCO_3 but on day 2 for 0% CaCO_3 . Note that the negative signs indicated that NH_3 was released from spring wheat seeds in the first day of germination.

For the 10% CaCO_3 soil sorption of NH_3 was lowest for APP with essentially negligible amounts after day 3. MAP produced a 3-day peak twice as high as APP but less than DAP. Also, the reduction to low levels occurred by day 4 for MAP, day 6 for DAP, but for UAP, the NH_3 sorption for day 6 still exceeded the 3-day peak even for DAP.

Table 9. Mean NH_3 production from 4 ammonium phosphates and NH_3 sorption by spring wheat seed or seedling in diffusion soil can for 6 days. Wheeler sil, 10.05% CaCO_3 , and Amsterdam sil, 0% CaCO_3 .¹

Fert. & Rate	NH ₃ system ²	Wheeler sil, 10.05% CaCO ₃						Amsterdam sil, 0% CaCO ₃					
		Days						Days					
		1	2	3	4	5	6	1	2	3	4	5	6
mg-N/100g soil		-----NH ₃ , µg/100g soil-----											
<u>MAP (11-48-0)</u>													
216	No seed	.40	.53	1.15	.21	.16	.11	.03	.02	.02	0	0	0
	with seed	.61	.10	.50	.08	.07	.08	.16	0	.01	0	0	0
	sorption	-.21	.43	.65	.13	.09	.03	-.13	.02	.01	0	0	0
<u>DAP (18-46-0)</u>													
216	No seed	1.28	1.41	1.83	1.51	1.16	.41	.27	.10	.08	.05	.06	.05
	with seed	1.51	.88	1.01	.98	.81	.33	.49	.02	.07	.04	.05	.04
	sorption	-.23	.53	.82	.53	.35	.08	-.22	.08	.01	.01	.01	.01
<u>APP (15-62-0)</u>													
216	No seed	.31	.36	.58	.06	.11	.05	.01	.02	.02	0	0	0
	with seed	.66	.22	.26	.03	.05	.04	.14	.01	.02	0	0	0
	sorption	-.25	.14	.32	.03	.06	.01	-.13	.01	0	0	0	0
<u>UAP (24-42-0)</u>													
216	No seed	1.86	2.43	5.88	4.18	3.56	2.08	.90	1.98	3.46	3.41	3.90	2.38
	with seed	2.08	1.14	2.47	1.79	1.88	1.17	1.12	.80	2.38	2.87	3.46	2.06
	sorption	-.22	1.29	3.41	2.39	1.68	.91	-.22	1.18	1.08	.54	.44	.32

¹ Each measured value is mean of 3 soil moisture levels and 2 reps.

² NH_3 measured H_3BO_3 in 2 systems--no seed & with seed. Sorption calculated by difference. Complete sorption data are in Appendix tables 11, 12, and 13.

LSD(.05) Sorption = 0.06 $\mu\text{g}/100\text{g}$ soil.

The data of plant growth in summarized form are presented in Table 10. Diammonium phosphate was applied to Amsterdam sil soil, 9.2% CaCO_3 , at rates of zero, 56, 112, and 224 mg-N/100g soil in a diffusion can closed system. Spring wheat seeds were planted and water was added to make soil moisture levels of 15%, 20%, 25% and 30%. Each figure is the average of 4 moisture levels and of 2 replications. Analysis of variance of these data showed significant differences in NH_3 concentrations due to fertilizer treatment. High concentration of NH_3 in seedlings affected plant development of both radicles and coleoptiles.

Table 10. Mean effects of NH_3 from DAP on spring wheat seed or seedling development in soil can--closed system for 6 days and 4 moisture levels. Amsterdam sil, 9.20% CaCO_3 .¹

N-Rate/ 100g soil	NH_3 conc/ seed or seedling	Mean		Mean Coleoptile/ seedling length
		Radicle/seedling No.	Length	
mg	ppm- NH_3	#	cm	cm
0	.111	3.9	9.8	12.7
56	.284	3.2	4.3	7.0
112	.530	1.2	.2	1.1
224	.895	-----No Germination-----		
LSD(.05)	.045	.5	.6	.9

¹ Complete data and analysis of variance are in Appendix Table 14.

Ammonia concentration of .284 ppm- NH_3 /100g plant fresh weight did not inhibit the number of radicle and coleoptile elongations;

however, it did reduce length. Above .530 ppm-NH₃/100g marked reductions in seedling growth and other symptoms of injury were observed. The root tips had a brown color as a burnt off appearance and the coleoptiles were stunted. Germination of wheat seeds was completely prevented when the mean concentration of NH₃ reached .895 ppm-NH₃. However, 0.771 ppm-NH₃/100g as shown in Appendix Table 14, actually prevented germination.

These evidences of damage to plants were observed to be about the same as described by Hunter and Rosenau (1967), and Colliver and Welch (1970) on the direct toxic effects of NH₃. They did not associate the damage to specific concentrations of NH₃, however.

Sorption of NH₃ for the 0% CaCO₃ soil (Table 9) was about none for APP and MAP. DAP had a small amount on day 2, with UAP also showing a peak on day 2 and reducing each day thereafter. Examining analysis of variance data in Appendix Table 13, the results show significance due to soils, fertilizer sources, and moisture levels.

The data show that NH₃ release and accumulation over time is probably very important as it relates to critical sensitive stages in the germination and seedling development processes of small grain crops. With reference to data in Tables 8 and 9, the greatest magnitude of NH₃ absorption by wheat seedlings was on the third day for all fertilizer treatment on the calcareous soil. Therefore, from the

standpoint of plant development, the third day is expected to be the most susceptible stage of spring wheat seedling development to NH_3 toxicity, if there is enough NH_3 produced by the fertilizer at that particular time. With the highly calcareous soil, maximum NH_3 is released during the third day for all four fertilizers.

The concentration of NH_3 that is required to be toxic to spring wheat seeds or seedlings was measured by the distillation technique described in the METHODS AND MATERIALS section. By this method it is not claimed that all the the NH_3 measured existed in the plants or seeds as nonionized NH_3 . However, the data should provide a basis for determining relative concentrations causing death or damage to plants.

It is pointed out that the NH_3 concentration of 0.8 ppm- NH_3 in the seeds, which completely prevented germination, was obtained by quantitative measurement directly from the plants, and the specific ranges are given for spring wheat seeds or seedlings. As indicated above, values given here might be high because of the procedure of heating, if in fact they are in error at all. There were no current data found in the literature for comparison of either specific NH_3 concentrations or plant species where measurements were reported as being of the absorbed NH_3 . However, different plant species may be tolerant to different levels of NH_3 toxicity. Allred and Ohlrogge, 1964; Bennett and Adams, 1970; Ensminger et al., 1965; Hunter and Rosenau, 1966; Openshaw and Frederick, 1970 did report different

partial pressures of NH_3 gas in the air, or soil air, which produced toxic effects for corn and cotton.

The degree of tolerance of some plants to NH_3 toxicity may vary depending upon some physiological process. Assimilation of NH_3 in the plant, then formation of new synthate such as asparagine as one of the mechanisms for reducing the level of NH_3 below toxic level, has been suggested by Prianschnikov (1922). Some plant species, or even varieties, may be more efficient than others in such processes.

II. Growth Chamber Experiments

A growth chamber experiment using a central composite rotatable design tested fertilizers, rates, soils, and initial moisture as affecting wheat germination and development. Modified treatments and design are presented in MATERIALS AND METHODS. The data given in complete detail in Appendix Table 15 were subjected to a multiple regression analysis. Variables and coefficients for setting of orthogonal components in the multiple regression were as follows:

Variable No.	Dependent Variable	Variable No.	Dependent Variable
1	Dry wt.	18	UAP (F_4)
2	Fresh wt.	19	Soil (S)
3	Normal germination	20	Linear Moist. (M_L)
4	Abnormal germination	21	Quadratic Moist (M_Q)
5	Dead	22	Block (B)
6	Radicle length	23	Block effect (B_E)
7	Coleoptile length	25	$F_1 \times S$
8	Number of roots	26	$F_2 \times S$
9	NH_3 in plant	27	$F_3 \times S$
10	Linear rate (R_L)	28	$F_4 \times S$
11	Quadratic rate (R_Q)	29	$R_L \times T_L$
12	Linear time (T_L)	30	$R_L \times T_Q$
13	Quadratic time (T_Q)	31	$T_L \times M_L$
15	MAP (F_1)	32	$R_Q \times T_Q$
16	DAP (F_2)	33	$R_Q \times M_Q$
17	APP (F_3)	34	$T_Q \times M_Q$

Comparison	No. of levels				
	1	2	3	4	5
R_L	-2	-1	0	1	2
R_Q	2	-1	-2	-1	2
T_L	-2	-1	0	1	2
T_Q	2	-1	-2	-1	2
M_L	-1	0	+1	--	--
M_Q	+1	-2	+1	--	--
S_1	1	--	--	--	--
S_2	-1	--	--	--	--
F_1	1	0	0	0	--
F_2	0	1	0	0	--
F_3	0	0	1	0	--
F_4	0	0	0	1	--

Multiple regression analyses were obtained for measurements of dry weight, fresh weight and NH_3 absorption. Data for root counts, for example, were not complete enough to subject to analysis. This was because of no roots the first date of harvesting and by 12 days there was such a mat of roots that counts were impossible. The response surface for any particular effect could be calculated from the resulting equations. For plant dry weight, the equation was:

$$\begin{aligned} \hat{Y} = & .4728 - .0098 R_L - .0094 R_Q + .0172 T_L + .0206 T_Q \\ & + .0619 F_1 + .0594 F_2 + .0782 F_3 + .0039 F_4 \\ & - .0002 S + .0060 M_L + .0069 M_Q. \end{aligned}$$

For NH_3 concentration the equation was:

$$\begin{aligned}\hat{Y} = & 3.7300 + 1.3490 R_L + .7138 R_Q - 1.2820 T_L \\ & + 2.2210 T_Q + 4.4600 F_1 + 5.5950 F_2 + 2.6660 F_3 \\ & + 8.9500 F_4 - .1603 S - .0692 M_L + 1.0560 M_Q.\end{aligned}$$

For plant fresh weight, the equation was:

$$\begin{aligned}\hat{Y} = & 2.567 - .2783 R_L - .1052 R_Q + .4598 T_L - .2450 T_Q \\ & - .1889 F_1 - .1800 F_2 + .0637 F_3 - .7503 F_4 + .0165 S \\ & + .2769 M_L - .0213 M_Q.\end{aligned}$$

Results of the stepwise analysis indicated some unusual results in that NH_3 absorption by plants was calculated as being not related to fertilizer rates. Observed data plotted on the calculated curves were not at all close. The effects of % CaCO_3 gave no indication of significance on any characteristics measured, although NH_3 absorption was observed as being significantly greater from soils of higher CaCO_3 .

There appear to be problems with the equations. The result is that, due to unresolved problems associated with the data processing and analysis, results of this growth chamber experiment are presented for further discussion based upon the actual observed data, even though comparisons are somewhat limited.

The data on plant fresh weight and NH_3 concentration in plants for 6 days and 12 days are presented in Tables 11 and 12, respectively.

Table 11. Plant fresh weight and NH_3 in plants from 4 ammonium phosphate fertilizers banded with seeds in growth chamber experiment on Amsterdam sil, 5.5% and 9.2% CaCO_3 . 6 days.¹

Fert. & Rate	% CaCO ₃ in soil			
	Fresh wt.		NH ₃ in Plant	
	5.5%	9.2%	5.5%	9.2%
N, mg/pot	-----g/pot-----		--µg/100g plant--	
<u>MAP (11-48-0)</u>				
54	2.18	2.26	7.31	8.91
162	1.64	1.86	10.93	12.79
Mean	1.91	2.06	9.12	10.85
<u>DAP (18-46-0)</u>				
54	1.84	2.41	9.72	10.28
162	1.38	1.89	13.88	14.06
Mean	1.61	2.15	11.80	12.17
<u>APP (15-62-0)</u>				
54	2.33	2.64	4.86	5.40
162	1.53	2.13	7.52	8.86
Mean	1.93	2.38	6.19	7.13
<u>UAP (24-42-0)</u>				
54	1.91	2.20	10.76	11.64
162	1.07	1.35	24.64	21.67
Mean	1.49	1.77	17.85	16.65

¹ Average over 2 moisture levels and 3 reps. Complete data are in Appendix Table 15.

Table 12. Plant fresh weight and NH_3 in plants from 4 ammonium phosphate fertilizers banded with seeds in growth chamber experiment on Amsterdam sil, 5.5% and 9.2% CaCO_3 . 12 days.¹

Fert. & Rate	% CaCO ₃ in soil			
	Fresh wt.		NH ₃ in plant	
	5.5%	9.2%	5.5%	9.2%
N, mg/pot	-----g/pot-----		--µg/100g plant--	
<u>MAP (11-48-0)</u>				
54	3.53	3.22	2.64	2.12
162	3.03	3.21	3.99	4.17
Mean	3.28	2.17	3.31	3.14
<u>DAP (18-46-0)</u>				
54	3.76	3.58	3.22	2.32
162	3.11	3.22	6.35	5.02
Mean	3.43	3.40	4.78	3.67
<u>APP (15-62-0)</u>				
54	4.08	4.03	1.88	1.64
162	3.67	3.92	3.18	1.28
Mean	3.87	3.97	2.53	1.46
<u>UAP (24-42-0)</u>				
54	3.27	2.91	4.09	4.26
162	1.88	2.12	8.20	12.07
Mean	2.57	2.51	6.14	8.16

¹ Average over 2 moisture levels and 3 reps. Complete data are in Appendix Table 15.

Each value is an average of 2 initial soil moisture levels and 3 replications. Data illustrate the effects of 4 ammonium phosphate fertilizer sources at 2 rates and for 2 soils. For the 5.5% CaCO_3 soil, the means of two rates for each ammonium phosphate fertilizer produced plant fresh weight responses in the yield order as follows:
 $\text{APP} = \text{MAP} > \text{DAP} > \text{UAP}$.

Fresh weight yield was larger for the higher CaCO_3 soil. Due to the % CaCO_3 content of the soils being not widely different, plant responses were not as large or as distinctly different as might have been expected if one soil were noncalcareous. Data from the second growth chamber experiment verify that a greater difference in CaCO_3 was desirable. Another problem was the difficulty in obtaining a uniform soil moisture. The process of adding water resulted in some soil puddling of the 5.5% CaCO_3 soil which probably caused detrimental plant growth effects. Anyway, yield effects from soils in this experiment were reversed from the more expected results obtained in the next experiment where a different method was used for adding soil moisture.

Effects of NH_3 from 4 ammonium phosphate fertilizers on plant growth were apparent. Figures 7 and 8 illustrate the magnitude of NH_3 absorption by plants. The terminal times for these two sets of treatments were 6 and 12 days. The data clearly demonstrated that the higher rate of fertilizer application, which produced more NH_3 ,

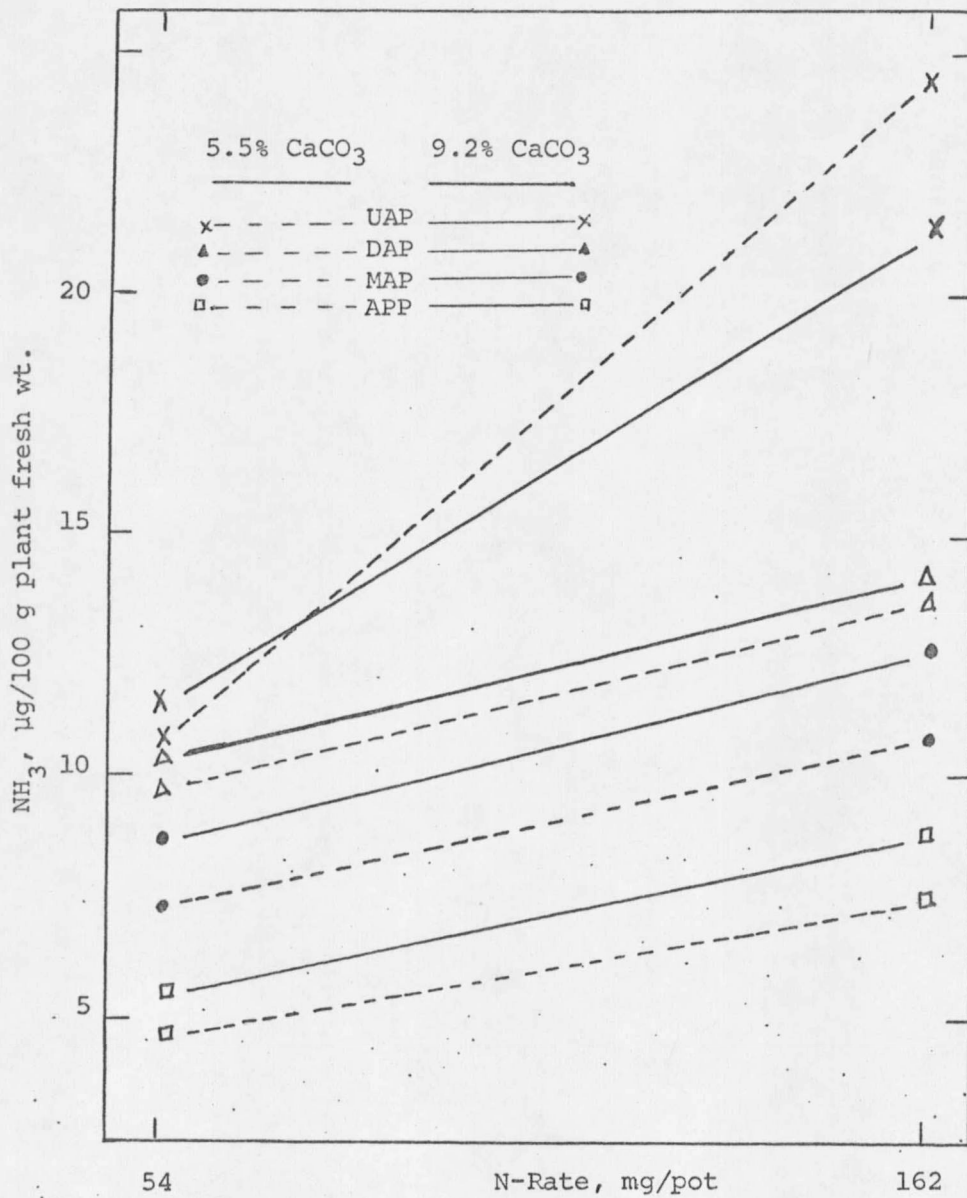


Figure 7. NH_3 concentration in plants as affected by MAP, DAP, APP, and UAP in growth chamber experiment. 6 days. Amsterdam sil, 5.5% and 9.2% CaCO_3 .

