

THESIS

NITRIFICATION SUPPRESSION AND SPINACH GROWTH
IN ALKALINE SOIL

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER OUR SUPERVISION BY CRAIG E. RIGGERT ENTITLED
NITRIFICATION SUPPRESSION AND SPINACH GROWTH IN
ALKALINE SOIL BE ACCEPTED AS FULFILLING IN PART
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

NITRIFICATION SUPPRESSION AND SPINACH GROWTH IN ALKALINE SOIL

A single at-planting application of $(\text{NH}_4)_2\text{SO}_4$ with and without nitrapyrin,^z [2-chloro-6-(trichloromethyl) pyridine], was evaluated on spinach in alkaline soil in 2 field experiments and 1 greenhouse experiment. Parameters measured were soil NH_4 -N and NO_3 -N, spinach yield, leaf color and leaf P, Fe and Zn. Nitrapyrin was adsorbed onto $(\text{NH}_4)_2\text{SO}_4$ crystals then applied in a band at planting in the field experiments. The first field experiment consisted of N application rates of 87.8 and 157.5 kg/ha and nitrapyrin rates of 0, 280 and 560 g/ha while the second field experiment included N application rates of 0, 45 and 90 kg/ha and nitrapyrin rates of 0, 140, 280 and 420 g/ha. Nitrapyrin (1.5 ppm - dry soil weight basis) was adsorbed onto $(\text{NH}_4)_2\text{SO}_4$ crystals and incorporated with soil in the greenhouse at N rates of 0, 400 and 800 mg/2 kg dry soil. Drainage was prevented in this greenhouse experiment.

Spinach yields were measured and leaf and soil samples were taken at harvest from the first field experiment and the greenhouse

^z N-SERVE®

experiment. Yields were measured and leaf and soil samples were taken 28, 42, 48 and 56 days from planting in the second field experiment.

Apparently little leaching occurred in the first field experiment and final yield was greatest when N applications included 280 g nitrapyrin/ha. This resulted in high soil concentrations of both NH_4 and NO_3 at harvest. Concentrated soil NH_4 resulting from prolonged nitrification suppression with as much as 560 g nitrapyrin/ha produced good plant growth on this alkaline soil. Leaching had apparently occurred in the second field experiment and increased yields with nitrapyrin 56 days from planting were attributed to increased soil N retention. Prolonged nitrification suppression from 1.5 ppm nitrapyrin in the greenhouse resulted in severely stunted plant growth along with other NH_4 toxicity symptoms.

Leaf color, which is important in determining market and nutritional quality of spinach, was darker green when $(\text{NH}_4)_2\text{SO}_4$ adsorbed with nitrapyrin was applied. This was measured during all 3 experiments. The dark green leaf color was measured and/or observed well before observed yield differences.

Leaf P, Fe and Zn concentrations were generally increased when NH_4 applications included nitrapyrin in this study. Leaf P concentrations increased the most while leaf Zn concentrations increased

the least. Increase in leaf P, Fe and Zn concentrations due to the addition of small amounts of nitrapyrin to NH_4 fertilizer may be an important consideration with regard to alkaline soils where P, Fe and Zn are often limiting.

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INTRODUCTION

Nitrogen is often the single most limiting factor in crop production. Crop production is becoming increasingly difficult with less N fertilizer available as well as increased costs. The demand for N by a crop such as spinach is intense over a short time span. This shallow rooted, fast growing species reaches a critical demand period early and only continuous N availability results in high yields of quality spinach. In the arid West frequent surface irrigations are necessary, particularly during seed germination. Nitrogen must be applied at frequent intervals and in relatively large amounts to compensate for N leaching losses due to intensive irrigation practices. In some areas of the United States rainfall results in severe leaching of N. One of the major priorities in agricultural research must be to develop new ways to increase the efficiency of N applications.

During the growing season, N is converted to and maintained in the soil as NO_3 , an extremely mobile form of N that results in low N application efficiency. Soils have a greater capacity to retain N when it is in the NH_4 form, however, NH_4 is rapidly nitrified to NO_3 by biological processes. The efficiency of N applications can be increased with the retention of soil N in the NH_4 form by suppressing nitrification. The efficiency of N applications with nitrification suppression also depends on the ability of the crop to utilize NH_4 .

This study investigates the growth of spinach under suppressed nitrification conditions.

The color of spinach is an important element of marketing and nutritional quality. The darker the green color of spinach, the greater is its market value. Darker green tissue indicates higher nutritional value in that beta-carotene, pro-vitamin A, increases concomitantly with green color. Green tissue color is also related to crop nutrition with deficiencies of N and various micronutrients resulting in lighter green leaf color. Previous studies indicate that plants grown with NH_4 exhibit darker green leaf color than plants grown with NO_3 . This study investigates spinach leaf color under suppressed nitrification conditions.

The availability of essential elements, such as P, Fe and Zn, is often reduced in alkaline soils. An NH_4 soil regime may increase the availability of these elements. This study investigates the effect of nitrification suppression on leaf concentrations of P, Fe and Zn.

Two field experiments and one greenhouse experiment were used to investigate the effects of nitrification suppression on yield, leaf color, and leaf concentrations of P, Fe and Zn with regard to spinach growing in alkaline soil. Soil NH_4 -N and NO_3 -N concentrations, which are the underlying elements from nitrification suppression that most greatly influence plant growth, were measured in all

3 experiments. The nitrification suppressant used throughout the study was nitrapyrin,^z [2-chloro-6-(trichloromethyl) pyridine].

^z Formulated and supplied by the Dow Chemical Company, Midland, Michigan. N-SERVE is the registered trade name.

LITERATURE REVIEW

Nitrification Suppression

For many years NO_3 was accepted as a good form of N for plant growth while NH_4 was considered an unsuitable form. Retention of NH_4 in the soil is becoming increasingly studied as these speculations are being refuted. Ammonium does not suffer loss through leaching and denitrification as does NO_3 . In a natural soil, Nitrosomonas bacteria oxidize NH_4 to NO_2 which is further oxidized to NO_3 by Nitrobacter bacteria. This nitrification process is extremely rapid and NH_4 may be completely oxidized 4 weeks after application (36).

Nitrifying bacteria are extremely sensitive to soil fumigation. The broad spectrum soil fumigant D-D (116) and the nematicidal fumigant Telone (138) applied at recommended rates inhibited nitrification for 8 weeks. Picloram at 1000 ppm suppressed nitrification by 50% (38).

Chemicals have been developed that are specifically toxic to Nitrosomonas sp. Prasad, Rajale and Lakhdive (100) in 1970 reviewed research concerning chemicals that specifically suppress nitrification. Thiourea, methionine, dicyandiamide and a number of urethanes suppress nitrification but none of these are as active and

specific towards Nitrosomonas sp as the nitrification suppressant developed in 1962 by Dow Chemical Company, 2-chloro-6-(trichloromethyl) pyridine, with the trade name N-SERVE (95). This pyridine compound, also known as nitrapyrin, has a low toxicity to Nitrobacter organisms, to most fungi and other bacteria, to algae and to many plant seedlings (36, 37, 38, 107). Plants begin to exhibit phytotoxicity symptoms at nitrapyrin rates of 12.5 to 100 ppm (dry soil weight basis) (36, 82).

Goring, who is responsible for the development of nitrapyrin, indicated in 1962 that nitrapyrin might be widely used to decrease N losses. The displacement of N from the root zone during critical growing periods might be avoided, timing of N applications would be less critical and the number of N applications for some crops might be reduced with nitrapyrin (36).

The minimum concentration of nitrapyrin required to delay nitrification for at least 6 weeks varies from as low as 0.05 ppm to as high as 20 ppm, depending on soil and environmental conditions. Soils high in organic matter and high pH soils require greater concentrations of nitrapyrin to suppress nitrification (36). One ppm nitrapyrin with $(\text{NH}_4)_2\text{SO}_4$ delayed nitrification for 30 days in Midwestern soils (104) and 120 days in a Southeastern soil (16). Turner, Warren and Andriessen (124) reported that nitrapyrin gave partial control of nitrification at rates of active ingredient varying from

0.5 to 2.0% of the fertilizer N. Increasing concentrations of nitrapyrin delay nitrification for longer periods of time (36, 52, 124).

Suppressing nitrification with fall-applied $(\text{NH}_4)_2\text{SO}_4$ decreased the amount of N lost by preventing downward N movement in the soil profile (33, 51). Nitrapyrin is effective in reducing N losses during all seasons of the year (52).

The NH_4 fertilizer most often used with nitrification suppression studies has been NH_4 salts, however, nitrapyrin has been reported to be extremely effective in suppressing nitrification with anhydrous NH_3 (52, 56, 58, 85, 95, 96). In fertilizer comparison studies, nitrapyrin controlled nitrification with $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , aqua NH_3 and anhydrous NH_3 (37, 124). Nitrapyrin also controls nitrification with urea (37, 123).

A single application of N for the production of leafy vegetables (85) and cotton, sweet corn, sugarbeets, cane, rice, and spinach (123) without additional side-dress N applications was possible when nitrapyrin was used to suppress nitrification. Nitrapyrin was applied with several NH_4 fertilizers and increased yields of cotton, sweet corn, and sugarbeets (114). Maximum corn (58) and wheat (50) yields have been produced from lower N applications with nitrapyrin. All these investigators attributed their results to increased soil N retention by the delayed conversion of NH_4 to NO_3 .

NH_4 -N Nutrition

Nitrate must be reduced to NH_4 before it can be assimilated into organic N forms. Ammonium has possible nutritive advantages over NO_3 because of the lack of an energy consuming reductive step, however, an internal accumulation of NH_4 results in poor plant growth. Plants can tolerate relatively high accumulations of NO_3 and the NO_3 reduction process is metabolically regulated to prevent toxic NH_4 accumulations. The uptake and assimilation of NH_4 is uncontrolled and toxic tissue accumulations of NH_4 are possible when high concentrations of NH_4 are available to the plant. Beans exhibit NH_4 toxicity symptoms of curling and burning of the leaf margins and necrosis of the laminae (7). High internal NH_4 accumulations produce lesions on tomato stem and leaf tissue (4, 129). Bean, cucumber and pea exhibited reduced growth rates, wilting, marginal necrosis and interveinal chlorosis of terminal leaves with concentrated NH_4 nutrition (74). Ammonium may inhibit ATP production and photosynthetic electron transport systems (105). Root symptoms attributed to NH_4 toxicity are dark coloration and decreased size (12, 63, 74). Severe plant wilt under moisture stress with toxic accumulations of NH_4 (12) may be due to membrane impairment or structural damage, a decrease in osmotic potential, or prolonged stomatal closure (101). To prevent NH_4 toxicity, NH_4 must be efficiently combined into organic N forms.

The primary organic N forms assimilated from NH_4 are glutamine and asparagine (2, 105, 135). Glutamine may be the primary N storage amide for most plants. Approximately 80% of NH_4 applied to barley was combined into glutamine (141). There is a close association between the synthesis of amides and the plant's respiration rate (141). An increase in O_2 consumption has been observed with NH_4 nutrition (5, 60). In plants undergoing NH_4 nutrition, carbohydrates break down during respiration into carbon linkages with subsequent amination and CO_2 release (76, 115). Extremely high NH_4 concentrations in the medium results in rapid depletion of carbohydrates reducing plant growth.

Tomato roots grow best with NO_3 at a pH of 4.6 to 5.0 and with NH_4 at a pH of 7.0 or greater (108). This is consistent with other works that indicated NO_3 to be a good N form at acid pH and NH_4 at slightly alkaline pH (34, 136). Ammonium assimilation is more greatly affected than uptake by pH of the medium or solution as indicated by rapid NH_4 absorption over a very wide pH range (108). A large number of studies have reported increased solution acidity with NH_4 nutrition and conversely more alkalinity with NO_3 nutrition (6, 27, 61, 63, 74, 76, 120). Anions and cations are absorbed independent of one another and H or OH ions are released from the plant to maintain internal electrochemical neutrality (15, 26, 60). Increase

in the uptake of NH_4 cation results in increased release of H ions reducing media pH.

Metabolic reduction of NO_3 raises cellular pH while assimilation of NH_4 into organic N forms decreases cellular pH (19, 26, 55, 62). Plants that receive NH_4 have 2 acidifying internal assimilative reactions (105). Ammonium combines with glutamic acid to form glutamine and with CO_2 and ATP to form carbamyl phosphate. Both reactions release H ions that must be combined within the cell or released into the solution media. Recently, Hodges (49) has developed a model that may further explain increased solution acidity with NH_4 nutrition. As NH_4 or any other cation is absorbed, ATPase is activated in the plasma membrane dissociating water and a pH gradient is established across the membrane with H ions accumulating outside the cell. Acidity is further increased by the exchange of internal H ions for NH_4 from the cation carrier.

Many NH_4 nutrition studies have involved solution experiments because of the inability to maintain NH_4 in a soil due to nitrification. Solution experiments have been criticized because the true effect of the N form on plant growth is complicated by changes in solution pH (62, 76, 139). Generally, solutions have a very poor buffering capacity as compared with soils.

Soils have a greater capacity to buffer bulk pH changes than to buffer pH changes within a few millimeters of the root (90, 91). There

is a large pH difference between bulk soil solution and the rhizosphere (103, 110, 111). Bulk soil pH with respect to NH_4 and NO_3 nutrition remained relatively unchanged after 6 weeks, while rhizosphere pH with NH_4 nutrition was 1.5 pH units lower than the rhizosphere pH with NO_3 nutrition (111). Rhizosphere pH changes with NH_4 and NO_3 nutrition were buffered with additions or presence of free lime.

Tomato seedlings (139) and field peas (135) have been shown to grow well in concentrated NH_4 solutions with additions of lime which maintained solution pH at a slightly alkaline level. Maynard and Barker (74) reported that growth of bean, sweet corn and pea in an unbuffered NH_4 solution was about 50% that of plants grown in NO_3 solutions. When lime was added to the solutions and a neutral pH maintained, plant growth with NH_4 was equal to plant growth with NO_3 (7, 108, 134). Unbuffered concentrated NH_4 solution resulted in protein degradation and toxic NH_4 accumulation within the plant (5, 62). Ammonium is more readily combined into organic N forms at a buffered alkaline pH and the level of free NH_4 in the plant is not sufficient to induce protein degradation. Symptoms of NH_4 toxicity have been delayed when carbonate sources were added to concentrated NH_4 solutions and high pH maintained (6). The metabolic pathways of NH_4 assimilation seem to be pH sensitive and more effective at neutral to alkaline pH.

The availability of K and rate of K uptake influences plant utilization of NH_4 . Potassium plays an important role in protein synthesis. Ammonium and NO_3 caused an accumulation of amides and amino acids with a loss in protein when K was deficient (129). Ammonium nutrition produced lowest levels of plant protein in the absence of K. Ammonium in concentration drastically reduced K uptake (22) through competition for absorption carrier sites (139). Ammonium substitution for K during absorption will result in poor, unstable protein structure. Additions of K to NH_4 solutions have increased the utilization of NH_4 by grasses (71) and also reduced stem lesions in tomato (4).

There is a close balance between cations and anions in plant tissue (10). Tissue produced with NH_4 has lower concentrations of inorganic cations and higher concentrations of inorganic anions as compared to tissue produced with NO_3 and the concentration of organic acids required to balance ionic differences within the plant is much less (24, 60, 63, 122). Extremely low levels of organic acids resulting from concentrated NH_4 nutrition limit the rate of other important metabolic processes (136, 139). Tomato (45, 64, 122) and sugarbeet (45, 122) exhibited poorer growth and lower organic acid content with NH_4 nutrition. In extensive study, DeWit, Dijkshoorn and Noggle (25) indicated that poor plant growth with NH_4 is due to stress on normal organic acid content.

The range of organic acids within all plants is extremely variable. When plants are forced to attain an organic acid level widely different from normal, a depression in growth can be anticipated, however, plants are capable of controlling their metabolic processes, including growth rate, over a very wide organic acid range (122). Organic acid content lower than normal has been observed in corn without yield reductions (66). Lower organic acid levels have been observed in wheat (64) and rice (54) with an increase in yields. The use of plant tissue organic acid levels to explain plant growth with NH_4 may not be valid.

NH_4 -N vs. NO_3 -N Nutrition

Ammonium can be absorbed (9, 68) and assimilated (32) much faster than NO_3 . Barker, Volk and Jackson (5) indicate a faster assimilation rate of NH_4 than NO_3 by bean plants with 46% of absorbed NH_4 combined into organic N while only 28% of the NO_3 was assimilated. Ammonium may inhibit NO_3 reduction (32, 54, 92, 97, 106, 109). Slight reductions in carbohydrate availability may account for disproportionately large reductions in NO_3 assimilation (115). When both NH_4 and NO_3 are available to the plant, NH_4 is absorbed and assimilated at a faster rate than NO_3 lowering carbohydrate reserves and thus decreasing NO_3 reduction rate.

Ammonium may be the best N form for plant growth when N is limiting (22, 71, 106). Radish growth was increased with NH_4 -N solution concentrations up to 17 ppm while lettuce growth increased with NH_4 -N solution concentrations up to 36 ppm (133). Higher NH_4 -N solution concentrations reduced radish and lettuce growth. Harada, Takaki and Yamada (45) reported that tomato growth from solutions with either NH_4 or NO_3 was the same at low N levels, but NO_3 produced best growth at higher N levels. Warncke and Barber (131) demonstrated that maximum dry matter production of corn at a N solution of 67 μM was best with an NH_4 -N to NO_3 -N ratio of 2.46 while at a N concentration of 303 μM a ratio of .17 was best. Higher levels of N nutrition require a majority of N to be in the NO_3 form because of possible NH_4 toxicity with high NH_4 concentrations. Low NH_4 concentrations result in rapid plant growth without toxic NH_4 accumulations.

Plants that grow well in acid soil, such as pine (75, 126), cranberry (40), blueberry (20, 121) and other calcifuge species (34) show a preference for NH_4 . Nitrifying bacteria are absent or in numbers too small to carry on the nitrification process in acid conditions. Ammonium may therefore be the natural N form for plants that thrive in reduced acid conditions and perhaps their metabolic pathways have been developed to utilize NH_4 in concentration. Rice grows best with an NH_4 -N source (54, 99, 109). Rice is partially cultivated under

waterlogged conditions where N predominates in the NH_4 form. Rice is another example of species adaptability to N form.

Some species have greater capacities to detoxify NH_4 than others. Pea responds better to NH_4 nutrition than cucumber (3) and carrot and sweet potato assimilate NH_4 more effectively than radish (29) because of greater capacities to produce amides. Onion grew well with NH_4 on fumigated soils (138), possibly because of greater carbohydrate reserves for NH_4 assimilation (74). Maize can tolerate high levels of NH_4 because of a large capacity to detoxify and store NH_4 in organic N forms (2).

Dicotyledonous crops often yield best with NO_3 while monocot grasses yield best with NH_4 (137). Spratt and Gasser (113) used nitrapyrin to compare the growth of wheat, ryegrass and kale with either NH_4 or NO_3 . Spratt and Gasser reported wheat and ryegrass to prefer NH_4 , particularly at early growth stages. Kale showed no preference for NH_4 or NO_3 approximately 2/3 (90 days) through the growing season after which NO_3 was the preferred N form. Other species also appear to have changing preferences for NH_4 and NO_3 at various growth stages (47, 76). In an early study (87), plants up to 5 weeks in age grew best and absorbed more N from NH_4 than NO_3 . After 5 weeks the plants grew best with NO_3 (78). Wheat (18, 112, 113) and maize (70, 131) grew well with concentrated NH_4 during early growth stages. Schrader et al. (106) demonstrated that early corn

growth was best with a NO_3 -N to NH_4 -N solution ratio 1:3 while later growth was best with a 1:1 ratio.

A combination of NO_3 and NH_4 often produces best plant growth. A 5:1 ratio of NO_3 -N to NH_4 -N in solution increased wheat yields 50% over wheat grown with NO_3 alone (22). Wheat cells (127), sunflower (135) and tomato (77) in solution experiments and tobacco (39, 102) in fumigated soils produced best growth with a combination of NO_3 and NH_4 . Pea and cucumber (3, 140) and greenhouse spinach (83) yields with combinations of NO_3 and NH_4 were comparable to yields with NO_3 alone. Cox and Reisenauer (22) indicated that low concentrations of NH_4 with higher concentrations of NO_3 may increase yields because of reduced energy required to utilize NH_4 . This may increase photosynthetic capacity and therefore increase protein synthesis. Plant growth is limited with all NH_4 nutrition by NH_4 toxicity and is also limited with all NO_3 nutrition by NO_3 reduction. Percent protein (106, 112, 134, 135) and NO_3 reductase activity (106) have been increased by combinations of NH_4 and NO_3 . It appears that with a portion of the N supplied and maintained as NH_4 , plant growth and development may be accelerated.

NH_4 -N Nutrition and Leaf Color

Dark green leaf color has been observed with cranberry (40), pines (57), and corn (15, 65) when grown with NH_4 . Jannsen (56) observed darker green leaf color of corn grown under suppressed nitrification conditions. Dark green tissue color often accompanies NH_4 toxicity symptoms (45, 63, 74).

Green color is an important market and nutritional quality of vegetables, particularly spinach. Carotenoid content is correlated with the green color of hays and silage (8). Beta-carotene, pro-vitamin A, has been related to green leaf color (125). Spinach color is highly dependent on N availability (41, 73). Chlorotic spinach leaf color has been associated with high pH and low levels of available micronutrients (31, 48).

Machold (69) observed that tomato grown with NO_3 often exhibited a lighter green leaf color than tomato grown with NH_4 . Ammonium raised the chlorophyll content 100 to 200%. Machold felt that NH_4 and NO_3 create different redox potentials within plants. The oxidized condition with NO_3 probably resulted in blockage of Fe while reduced conditions with NH_4 increased Fe utilization.

NH_4 -N Nutrition and Tissue P Concentration

Western alkaline soils often have high concentrations of total P, however, a majority of the P is precipitated in relatively insoluble forms often creating deficiency conditions. High concentrations of Ca in alkaline soils result in P combinations of insoluble calcium phosphates (14, 119). Ammonium increases the availability and uptake of P (24, 28, 59, 89, 112, 121).

Nitrogen is required in large amounts and rapid absorption of NH_4 stimulates the uptake of anions because of the plants need to maintain internal ionic equilibrium (15, 23, 60, 88). The ion absorption model by Hodges (49) illustrates that greater cation uptake stimulates the anion absorption mechanism. Since P is absorbed by plants in anionic form, plant P concentrations are increased with NH_4 nutrition.

Some investigators have attributed the effect of NH_4 on plant growth as a partial reason for increased P absorption. Ammonium nutrition increased root growth resulting in greater absorptive root surfaces and has been associated with increased P uptake (43, 44, 53, 79, 80, 89). An increase in top growth with NH_4 creates concentration gradients that influence roots to absorb P, as well as other nutrients, more efficiently (43, 44). Major alterations in plant metabolism with NH_4 may be responsible for increased P absorption (22, 53, 79). Ammonium may influence the movement of P across

the root symplast and increase the rate at which the P-carrier complex releases P into the xylem (66).

Hydrogen ions produced and released with NH_4 nutrition seem to be the major influence on P absorption. Phosphorous is absorbed best from slightly acid solutions. The H_2PO_4^- form predominates at acid pH while $\text{HPO}_4^{=}$ predominates at more alkaline pH (46). The H_2PO_4^- ion is preferred by plants and the increased acidity with NH_4 nutrition allows this orthophosphate form to predominate in solution (55, 142). Increased acidity around the root with NH_4 nutrition also solubilizes calcium phosphates (15, 44, 53, 72, 81). While tissue P concentrations are poorly correlated with the bulk soil pH, P concentrations in tissue correlate very well with the rhizosphere pH(103). Lower pH at the soil-root interface (rhizosphere) resulting from NH_4 nutrition increases the absorption of P (72, 79, 81, 90).

NH_4 -N Nutrition and Tissue Fe and Zn Concentration

Iron and Zn can form precipitates with oxides, hydroxides, carbonates and sulfides at high pH. Iron and Zn are also adsorbed by clay minerals (14, 119). With an increase in H ion concentration, precipitates solubilize and Fe and Zn become displaced from exchange sites making them more available to the plant. Increases in tissue concentrations of Fe (30, 31, 42) and Zn (21, 30, 35, 117, 128, 132) have been attributed exclusively to lower media pH.

Tissue concentrations of some of the heavy micronutrient cations are increased with NH_4 nutrition although tissue concentrations of macronutrient cations are depressed (112). Increased concentration of H ions in the media solution with NH_4 nutrition is cited as the major reason for increases in Fe and Zn absorption (20, 35, 90, 91, 121). Reductions of rhizosphere pH dissolve $\text{Fe}(\text{OH})_3$ and other relatively insoluble Fe forms increasing the availability of Fe to plants (91). Brown and Jones (19) point out that roots naturally release "reductants" that reduce ferric forms and the absorption and translocation of Fe increased. They state that release of these "reductants" may be stimulated by lower rhizosphere pH.

Increased Zn absorption has been observed at high pH with NH_4 nutrition and it has been hypothesized that an NH_3 -Zn complex is responsible for increasing the availability of soil Zn (35, 65). Lauer (65) has shown this NH_3 -Zn complex to exist but indicates that the major effect of NH_4 on Zn absorption still appears to be due to increasing H ion concentrations in the rhizosphere.

METHODS AND MATERIALS

Field Experiment - 1974

The location of this field experiment was the San Luis Valley Research Center near Center, Colorado. The climate in this area is well adapted for fresh market spinach production. The field chosen had been planted to wheat the previous year. Table 1 lists the soil's chemical and physical characteristics. All important nutrients were available in adequate quantities except $\text{NO}_3\text{-N}$ and P. Phosphorous was broadcast at a rate of 250 kg P/ha and incorporated into the soil prior to planting and treatment application.

This experiment consisted of a 2 X 3 factorial arrangement with 2 N and 3 nitrapyrin application rates. The ammoniacal source was fertilizer grade crystalline $(\text{NH}_4)_2\text{SO}_4$ and was applied at N rates of 78.8 and 157.5 kg/ha. Nitrapyrin^z was applied at 0, 280 and 560 g/ha.

Ammonium sulfate crystals were used as a carrier for nitrapyrin. A measured amount of $(\text{NH}_4)_2\text{SO}_4$ was placed in a twin-shell blender, the corresponding amount of nitrapyrin gradually released from a buret onto the crystals, and the combination mixed for 30

^z N-SERVE 24[®], Toluene carrier, active ingredient nitrapyrin - 22.2% Lot no. WP 10314-1

Table 1. Chemical and physical characteristics^z of the soil from the 1974 field experiment.

Texture (feel)	Sandy loam
pH (paste)	7.5
CaCO ₃ (%)	1.1
Total soluble salts (mmhos/cm)	1.1
Organic matter (%)	0.7
NO ₃ -N (ppm)	9.8
Available P (ppm)	10.5
Available K (ppm)	245
DTPA Ext. Zn (ppm)	0.97
DTPA Ext. Fe (ppm)	11.3

^z CaCO₃ - acid-neutralization determination

Total soluble salts - filtered extract from saturated soil paste was measured for conductivity

Organic matter - potassium dichromate colorimetric determination

NO₃-N - phenoldisulfonic determination

Available P - ascorbic acid determination

Available K - ammonium acetate extraction and flame photometry

Zn and Fe - DTPA extraction and atomic absorption spectrophotometry

minutes. The N-nitrapyrin treatments were placed in high density polyethylene buckets, sealed, and stored at 4°C until application to prevent possible volatilization of nitrapyrin.

Seed beds 1 m wide were formed prior to planting and treatment application. Seed was planted and treatments applied simultaneously in 2-row plots, 6 m long, on June 12. A randomized complete block design with 8 replications was employed. Treatments were banded 5 cm below and 5 cm to the irrigation furrow side of the seed row. The spinach cultivar was Bloomsdale Long Standing, a variety that has frequently matured in 45 days in the San Luis Valley. Spinach was planted at a seeding rate of 9 kg/ha at an approximate depth of 1 to 1.5 cm. Frequency of irrigations was determined from daily Bellani plate (atmometer) readings (86). The field was cultivated and hand weeded twice during the experiment and sprayed once with Malathion to control insects. Visual observations of treatment differences were recorded throughout the experiment.

Plots were harvested July 31, 49 days from planting. Buffer strips 1.5 m long were removed from the ends of each plot leaving the middle 3 m for yield measurement and sampling. Plants were cut just above the crown and the total weight and number of plants from each 3 m double row plot was recorded. Spinach tissue from each plot was placed in plastic bags, sealed and immediately placed in a cooler at 4°C. Tissue was later packed with ice in polystyrene

coolers and transported to Fort Collins for color measurement and drying.

Spinach was removed from the cooler, washed and rinsed in cold water, then laid on paper toweling to dry. Color of recently matured leaves was determined from each sample and the tissue put in brown paper bags which were placed in a forced-draft drying oven at 70°C for 48 hours. Oven-dry tissue was ground with a stainless steel Wiley mill through a 40-mesh stainless steel screen. Ground tissue samples were sent to Agrico Chemical Company Laboratory, Washington Court House, Ohio, and analyzed for a variety of elements including P, Fe and Zn. Phosphorous and Fe were determined by emission spectrographic analysis and Zn determined by atomic absorption spectrophotometry.

A reflectance meter, developed by Ennis and Associates of Riverside, California, was used to determine leaf color (85, 130). The meter utilizes a sensing unit which contains a light source and a photocell that measures light reflected from the leaf surface at a wavelength of 660 nm. The instrument indicated amount of light absorbed by the leaf. The lower the level of reflected light or the greater the level of light absorbed, the darker green the tissue color. The following is the procedure developed to determine spinach leaf color:

1. Tissue samples were kept in a cooler prior to color measurement to prevent wilting.
2. Ten recently matured leaves were randomly chosen from the sample.
3. The upper surface of each leaf was used to measure the level of absorbed light.
4. For standardization, an area in the middle of the laminae, just to the right of the midrib (with the petiole towards the operator), was used for measurement.
5. The sensing unit of the reflectance meter was placed on the smoothest portion of the area chosen.
6. Leaf light absorption measurements from the savoy spinach leaves were difficult and if the first reading was unstable, other attempts were made until a stable reading had been established.
7. A blank white index card was used for the zero reading. If required, the meter was readjusted to a zero setting after every ten measurements.

At harvest, 5 soil core samples, 0 to 30 cm, were randomly taken within each harvested row. The following day, soil samples from each plot were spread on brown paper in the greenhouse to air-dry for 48 hours. Soil samples were ground with a ceramic mortar

and pestle and stored at 4°C. Soil samples were sent to Agrico Chemical Company Laboratory for analysis which included NH₄-N and NO₃-N. Soil NO₃-N and NH₄-N concentrations were determined by specific ion electrode analysis.

Greenhouse Experiment 1974-1975

Soil used in this experiment was obtained from the USDA-ARS Central Plains Experiment Range near Nunn, Colorado. Physical and chemical characteristics of the soil are listed in Table 2.

Spinach was grown in number 10 cans lined with polyethylene bags 15 X 9 X 35 cm in size. Perlite was mixed with the soil (1/3 by volume) in order to improve the tilth and dilute the soil NO₃-N concentration. Each can contained the equivalent of 2 kg of medium dried at 105°C.

Soil pH is an important characteristic with respect to NH₄ nutrition and an objective of this experiment was to determine the effect of nitrification suppression on spinach growth in moderately low, moderately high and very high pH systems.

Acetic acid was added to a portion of soil to lower pH. Acetic acid solutions were mixed with small soil samples, placed on plastic in the greenhouse and dried, then soaked with water and dried several more times for stabilization. By trial and error it was found that after 10 days the pH from 2 kg soil in which 4 g acetic acid solution had been added stabilized at 6.5.

Table 2. Chemical and physical characteristics^Z of the soil used in the 1974-1975 greenhouse experiment.

Texture (feel)	Sandy loam
pH (paste)	7.7
CaCO ₃ (%)	0
Total soluble salts (mmhos/cm)	0.5
Organic matter (%)	0.8
NO ₃ -N (ppm)	17
Available P (ppm)	13
Available K (ppm)	425
DTPA Ext. Zn (ppm)	0.19
DTPA Ext. Fe (ppm)	2.0
Cation Exchange Capacity	9.2

^Z CaCO₃ - acid-neutralization determination

Total soluble salts - filtered extract from saturated soil paste was measured for conductivity

Organic matter - potassium dichromate colorimetric determination

NO₃-N - phenoldisulfonic determination

Available P - ascorbic acid determination

Available K - ammonium acetate extraction and flame photometry

Zn and Fe - DTPA extraction and atomic absorption spectrophotometry

Cation Exchange Capacity - NaOAc saturation and flame photometry

Calcium hydroxide was added to another portion of the soil to raise pH. The soil was non-calcareous, therefore to raise pH to approximately 8.3, 3% lime or an equivalent amount of $\text{Ca}(\text{OH})_2$ must be added to the soil. Calcium hydroxide was added to the soil at the rate of 44.1 g/2 kg soil. This treatment was also water soaked and dried several times on plastic film for stabilization. The pH of soil treated with $\text{Ca}(\text{OH})_2$ was very high at first, however, at the end of 10 days pH had stabilized at 8.3.

On December 6, 720 g (654 ml) of glacial acetic acid in dilute solution was mixed with 360 kg soil (dry weight equivalent) followed by the addition of perlite and designated Soil 1. Calcium hydroxide was added to soil at the rate of 7.94 kg/360 kg soil followed by the addition of perlite and designated Soil 3. Soil mixtures were placed on plastic film in the greenhouse for stabilization. Untreated soil-perlite mixture was labelled Soil 2.

A.C.S. certified crystalline $(\text{NH}_4)_2\text{SO}_4$ was the ammoniacal source and was applied at N rates of 0, 400 and 800 mg/2 kg (dry weight equivalent) of soil medium (118). Nitrapyrin^z was applied at 0 and 1.5 ppm (dry soil weight basis). A measured amount of $(\text{NH}_4)_2\text{SO}_4$ was placed in a gallon jar and the corresponding amount of nitrapyrin slowly added. The jar was sealed, placed in a twin-shell

^z Same N-SERVE[®] formulation used in the first experiment

blender and the contents mixed for 1 hour. Nitrapyrin was mixed with a measured amount of sand for the nitrapyrin-no N control treatment.

From December 16 to 22, N and nitrapyrin treatments were blended with soil-perlite medium and placed into polyethylene film lined cans. Phosphorous level of the original soil was low, therefore, 333 mg P as $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ was mixed with each 2 kg of medium (118). Ammonium sulfate and nitrapyrin treatments and CaHPO_4 was blended with 2 kg of medium in a twin-shell blender for 1 minute.

In summary, the greenhouse experiment consisted of a factorial combination of the following treatments: 1) Soil pH of 6.5, 7.7 or 8.3 2) N rates of 0, 400 or 800 mg/2 kg of medium 3) 0 or 1.5 ppm nitrapyrin. Cans were divided into 15 completely randomized blocks. Each block consisted of 36 cans, 2 from each treatment.

Spinach seed was planted into polystyrene trays^z and placed under a mist system on November 15. The spinach cultivar was Bloomsdale Long Standing treated with captan to prevent possible damping-off. Tray medium was the same as the untreated experimental medium. Two seeds were planted per tray compartment and thinned to 1 after emergence. Seedlings were showing N deficiency

^z Speedling trays, manufactured by Speedling Corp., Sun City, Florida.

symptoms 2 weeks after emergence and was corrected by 2 waterings with Hoagland number 2 solution (1/3 strength). Spinach seedlings similar in size and vigor and in the 4 to 6 leaf stage were removed from the polystyrene trays and transplanted into cans on December 23.

The amount of water supplied to the closed cans had to be monitored to prevent waterlogged conditions. Moisture was maintained near a tension of 0.3 bar in the cans. Soil-perlite mixture contained 19% moisture at 0.3 bar as determined from a previously constructed moisture tension curve. The amount of moisture required was determined on a weight basis. All cans were watered every 4 to 6 days, depending on environmental conditions. The first 2 waterings contained STEM (soluble trace element mixture) with concentrations of Mn (5.75 mg), Fe (5.35 mg), Cu (2.36 mg), Zn (3.16 mg), B (1.0 mg), Mo (0.04 mg) and S (10.69 mg) to supply plants with adequate concentrations of micronutrients.

Polyethylene tubing, 45 cm long, was placed at one side of each can 10 to 15 cm into the soil. Following the first 3 weeks when spinach was surface watered, water was added through the tubes to prevent heavy removal of N from the upper portions of the medium, to better distribute moisture through the soil system and to make watering easier when spinach covered the soil surface.

Day and night temperatures of the greenhouse were closely regulated and monitored to generate maximum spinach growth.

Daytime air temperatures ranged between 24 and 28°C while night temperatures ranged between 14 and 18°C.

Common whitefly and aphid problems were controlled until harvest with fumigation. An aphid infestation forced harvest on March 21, 88 days after transplanting.

Prior to harvest, leaf color was determined following the same procedure given in the 1974 field experiment except leaves remained on the plant. Light absorption was measured from 2 recently matured leaves per plant.

Plants were harvested, individually weighed, then placed in a paper bag with the plant from the duplicated treatment from the same block. Plants were dried in a forced-draft oven at 70°C for 48 hours then ground with a stainless steel Wiley mill through a 40-mesh stainless steel screen. Treatments were combined to form 5 rather than 15 samples because of small sample size. Each sample consisted of leaf tissue from 6 plants. Tissue was analyzed by Ag-Consultant Laboratory, Brighton, Colorado, for P, Fe and Zn. Phosphorous was determined colorimetrically with Barton's reagent and Fe and Zn determined by atomic absorption spectrophotometry.

Four soil cores, 0 to 15 cm, were taken from each can at harvest. Soil samples were spread on paper in the greenhouse to air-dry for 48 hours then stored for subsequent analysis. Soil samples were combined in the same manner as the tissue samples. Soil was

analyzed for pH from a soil paste with a glass-calomel electrode system (13) and NH_4 -N and NO_3 -N was determined with Orion specific ion electrodes and meter (1, 17, 93, 94).

Field Experiment - 1975

This field experiment was located at the San Luis Valley Research Center near Center, Colorado. The field had been planted to wheat the previous year. Table 3 lists the chemical and physical characteristics of this soil. Prior to planting and treatment application, a leaching procedure was used to lower residual soil NO_3 -N levels. A broadcast application of 250 kg P/ha was incorporated into the soil to supplement residual soil P.

The ammoniacal source was fertilizer grade crystalline $(\text{NH}_4)_2\text{SO}_4$ with N rates of 0, 45 and 90 kg/ha. Nitrapyrin^z rates were 0, 140, 280 and 420 g/ha. A measured amount of $(\text{NH}_4)_2\text{SO}_4$, or sand for the no N control treatments, was placed in the twin-shell blender and the corresponding amount of nitrapyrin added. Ammonium sulfate-nitrapyrin and sand-nitrapyrin combinations were mixed for 30 minutes, then placed in high density polyethylene buckets, sealed and stored at 4°C until application.

^z Same N-SERVE® formulation used in the previous experiments

Table 3. Chemical and physical characteristics^z of the soil from the 1975 field experiment.

Texture (feel)	Sandy loam
pH (paste)	7.9
CaCO ₃ (%)	0.9
Total soluble salts (mmhos/cm)	1.1
Organic matter (%)	1.6
NO ₃ -N (ppm)	40
Available P (ppm)	28
Available K (ppm)	380
DTPA Ext. Zn (ppm)	0.8
DTPA Ext. Fe (ppm)	6.0

^z CaCO₃ - acid-neutralization determination

Total soluble salts - filtered extract from saturated soil paste was measured for conductivity

Organic matter - potassium dichromate colorimetric determination

NO₃-N - phenoldisulfonic determination

Available P - ascorbic acid determination

Available K - ammonium acetate extraction and flame photometry

Zn and Fe - DTPA extraction and atomic absorption spectrophotometry

Seed was planted and treatments applied on June 12. Seed beds 1 m wide were formed prior to planting. Treatment plots were 4 rows wide and 7.5 m long. Treatments were banded 7.5 - 10 cm below and 5 cm to the irrigation furrow side of the seed row. The spinach cultivar was Bloomsdale Long Standing. Seed was treated with captan to prevent possible damping-off. All treatments were replicated 6 times and randomly assigned within blocks.

Gated pipe was placed at one end of the field with irrigation set for every furrow. Plots were irrigated approximately every 7 to 10 days depending on daily soil observations. Spinach was cultivated once, handweeded twice and required no pesticide applications. Visual observations regarding any treatment differences were recorded throughout the experiment.

A major objective of this experiment was to determine the effect of nitrification suppression on soil NH_4 and NO_3 levels, spinach yields, leaf color and leaf concentrations of P, Fe and Zn at various growth stages. Plots were sampled from 4 growth stages; July 10 (28 days from planting), July 24 (42 days from planting), July 30 (48 days from planting), and August 9 (56 days from planting). Yield data and tissue and soil samples were taken from the middle 2 rows of each 4-row plot to prevent possible error due to adjacent treatments. A 1.5 m buffer section was designated at both ends of each 7.5 m plot while the middle 4.5 m were used for sampling. One

and one half m were harvested from the west end of each plot at the first sample period, July 10. One m sections were harvested from each plot the remaining 3 sample periods. Spinach plants were cut just below the crown and the total weight and number of plants were recorded for each plot. Tissue was sealed in plastic bags and stored at 4°C. Tissue samples were packed with ice in polystyrene coolers for transportation to Fort Collins.

Recently matured leaves were removed from each sample, washed, drained, and placed in paper bags for drying. All tissue harvested, except from the first sample period, was measured for leaf color by the procedure previously described. Tissue was placed in a forced-draft drying oven at 70°C for 48 hours, ground with a stainless steel Wiley mill through a 40-mesh stainless steel screen, and sealed in glass vials. Tissue samples were sent to Ag-Consultant Laboratory and analyzed for P, Fe and Zn by methods previously described.

Soil samples were collected from each plot at 5 different times. Four soil cores, 0 to 30 cm, were taken from the middle 2 rows of each plot on July 1, 18 days from planting, and replications of each treatment were combined. Five soil cores, 0 to 30 cm, were randomly taken from each of the middle 2 rows of each plot where tissue had just been harvested from the other 4 sample periods which corresponded with tissue harvest dates. Soil samples were spread

on paper in the greenhouse the day following sampling and air-dried for 48 hours. Soil samples were ground by a bar mill to a mesh size of 10 and stored for analysis. Soil samples were analyzed for pH on a soil paste by a glass calomel electrode system (13) and NH_4 -N and NO_3 -N determined by Orion specific ion electrodes and meter (1, 17, 93, 94).

In summary, this experiment consisted of a factorial combination of the following factors: 1) N rates of 0, 45 or 90 kg/ha 2) Nitrapyrin rates of 0, 140, 280 or 420 g/ha 3) Sample times of 18, 28, 42, 48 or 56 days from planting for soil N measurements, sample times of 28, 52, 48 or 56 days from planting for spinach yields and leaf P, Fe and Zn concentration measurements, and sample times of 42, 48 or 56 days from planting for leaf color measurements.

RESULTS

Field Experiment - 1974

Soil NH₄-N, NO₃-N and pH

Nitrapyrin very effectively suppressed nitrification as indicated by soil NH₄-N and NO₃-N concentrations in Tables 4 and 5. The highest rate of nitrapyrin resulted in high NH₄-N and low NO₃-N concentrations in the soil at harvest indicating that nitrification was still being suppressed 49 days from application. Nitrification occurred relatively early within no nitrapyrin control treatments as indicated by high soil NO₃-N and low NH₄-N concentrations at harvest. High soil concentrations of NO₃-N and NH₄-N from the low nitrapyrin application rate indicated that although nitrification had at one time been suppressed, nitrification was active at harvest. Increasing the nitrapyrin application rate from 280 to 560 g/ha increased the soil NH₄-N level substantially at the high but not the low N application rate resulting in the significant NH₄-N nitrogen X nitrapyrin interaction. Nitrate-nitrogen levels were unexpectedly high in this experiment. It was apparent that relatively little leaching had occurred.

The original soil pH was measured at 7.5. At harvest, the pH of soils from N treatments without nitrapyrin was approximately 7.6.

Table 4. Mean soil $\text{NH}_4\text{-N}$ concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u> kg/ha		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		78.8	157.5		
<u>Nitrapyrin rate</u> g/ha	0	38	36	37	37
	280	82	91	86	115
	560	87	200	143	
Nitrogen		69	109		

Analysis of variance

Source	df	F values
Block	7	2.86 *
Nitrogen	1	6.69 *
Nitrapyrin	2	40.93 **
No Nitrapyrin vs. Nitrapyrin	1	56.78 **
280 vs. 560 g/ha	1	28.94 **
Nitrogen X Nitrapyrin	2	5.10 *
Error Mean Square	35	

* 5% significance level

** 1% significance level

Table 5. Mean soil NO₃-N concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u>		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		kg/ha			
		78.8	157.5		
	0	139	192	166	166
<u>Nitrapyrin rate</u> g/ha	280	104	132	118	100
	560	87	75	81	
Nitrogen		110	133		

Analysis of variance

Source	df	F values
Block	7	.75
Nitrogen	1	13.85 **
Nitrapyrin	2	41.42 **
No Nitrapyrin vs. Nitrapyrin	1	60.31 **
280 vs. 560 g/ha	1	22.53 **
Nitrogen X Nitrapyrin	2	1.28
Error Mean Square	35	

* 5% significance level

** 1% significance level

The soil pH from NH_4 -nitrapyrin applications at the nitrapyrin rate of 280 g/ha was 7.8 and 7.9 at 560 g nitrapyrin/ha. It appeared that the acidity created by nitrification from N applications without nitrapyrin lowered bulk soil pH more than NH_4 -nitrapyrin applications. Although a pH difference was observed, the relative difference was small. This soil apparently had a strong buffering capacity.

Spinach Yield

Ammonium-nitrapyrin applications produced greatest yields (Table 6), however, yields were less from the high rate of nitrapyrin than the low. Lower N rates seemed to produce larger yields than the higher rate. Seedlings that emerged from high N applications exhibited severe fertilizer burn symptoms and a large number of these plants died leaving several of the high N application plots with low plant populations. A plant count taken at harvest indicated that high N applications without nitrapyrin caused the most severe plant losses. There was no significant nitrogen X nitrapyrin interaction indicating that the effect of NH_4 -nitrapyrin applications on spinach yields was the same from both N application rates.

Leaf Color

Spinach leaf color differences were first observed 30 days from planting and the differences remained through harvest. Nitrogen applications with nitrapyrin exhibited darker green leaf color

Table 6. Mean spinach yields (MT/ha) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u> kg/ha		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		78.8	157.5		
<u>Nitrapyrin rate</u> g/ha	0	5.0	3.8	4.4	4.4
	280	7.1	6.8	7.0	6.3
	560	6.0	5.1	5.6	
Nitrogen		6.0	5.2		

Analysis of variance

Source	df	F values
Block	7	.64
Nitrogen	1	3.93
Nitrapyrin	2	11.75 **
No Nitrapyrin vs Nitrapyrin	1	16.26 **
280 vs. 560 g/ha	1	7.23 *
Nitrogen X Nitrapyrin	2	.38
Error Mean Square	35	

* 5% significance level

** 1% significance level

than N applications without nitrapyrin. Leaf light absorption measurements taken at harvest with the reflectance meter supported the visible leaf color differences (Table 7). Nitrogen application rate had no influence on leaf color nor did nitrapyrin application rate. The lack of a significant nitrogen X nitrapyrin interaction indicated that the effect of NH_4 -nitrapyrin applications on spinach leaf color was the same from both N application rates.

Leaf P, Fe and Zn Concentrations

Ammonium-nitrapyrin applications increased leaf P (Table 8) and Fe (Table 9) concentrations regardless of nitrapyrin rates. Leaf Zn concentrations were not increased by NH_4 -nitrapyrin applications (Table 10). Leaf P, Fe and Zn concentrations were highest from low rather than high N applications. Although Zn concentration was higher from low N applications, the relative increase was small. The effect of NH_4 -nitrapyrin applications on P concentration differs between N application rates as indicated by a significant nitrogen X nitrapyrin interaction. As nitrapyrin was increased from 280 to 560 g/ha, leaf P concentration from the low N application increased while P concentration from high N applications decreased. Although the nitrogen X nitrapyrin interaction relative to leaf Fe concentration was not significant, the trend was similar to leaf P concentrations.

Table 7. Mean leaf light absorption values (green color) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u>		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		kg/ha			
		78.8	157.5		
	0	.71	.71	.71	.71
<u>Nitrapyrin rate</u> g/ha	280	.72	.72	.72	.72
	560	.72	.73	.73	
Nitrogen		.72	.72		

Analysis of variance

Source	df	F values
Block	7	1.25
Nitrogen	1	.14
Nitrapyrin	2	3.27 *
No Nitrapyrin vs. Nitrapyrin	1	6.21 **
280 vs. 560 g/ha	1	.86
Nitrogen X Nitrapyrin	2	.27
Error Mean Square	35	

* 5% significance level

** 1% significance level

Table 8. Mean leaf P concentrations (%) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u> kg/ha		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		78.8	157.5		
	0	.47	.47	.47	.47
<u>Nitrapyrin rate</u> g/ha	280	.83	.51	.67	.69
	560	.76	.65	.71	
	Nitrogen	.69	.54		

Analysis of variance

Source	df	F values
Block	7	2.56 *
Nitrogen	1	9.56 **
Nitrapyrin	2	10.14 **
No Nitrapyrin vs. Nitrapyrin	1	20.00 **
280 vs. 560 g/ha	1	.80
Nitrogen X Nitrapyrin	2	4.04 *
Error Mean Square	35	

* 5% significance level

** 1% significance level

Table 9. Mean leaf Fe concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u> kg/ha		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		78.8	157.5		
	0	308	251	280	280
<u>Nitrapyrin rate</u> g/ha	280	384	317	351	346
	560	348	335	342	
Nitrogen		346	301		

Analysis of variance

Source	df	F values
Block	7	4.60 **
Nitrogen	1	4.18 *
Nitrapyrin	2	3.90 *
No Nitrapyrin vs. Nitrapyrin	1	7.70 **
280 vs. 560 g/ha	1	.11
Nitrogen X Nitrapyrin	2	.51
Error Mean Square	35	

* 5% significance level

** 1% significance level

Table 10. Mean leaf Zn concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications. Spinach field experiment - 1974.

		<u>Nitrogen rate</u> kg/ha		Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		78.8	157.5		
<u>Nitrapyrin rate</u> g/ha	0	41	39	40	40
	280	42	39	41	40
	560	41	39	40	40
Nitrogen		41	39		

Analysis of variance

Source	df	F values
Block	7	26.43 **
Nitrogen	1	7.31 **
Nitrapyrin	2	.38
No Nitrapyrin vs. Nitrapyrin	1	--
280 vs. 560 g/ha	1	--
Nitrogen X Nitrapyrin	2	.34
Error Mean Square	35	

* 5% significance level

** 1% significance level

Greenhouse Experiment 1974-1975

The pH of the soil-perlite medium treated with acetic acid (Soil 1) was 6.6 at transplanting. This soil system pH gradually increased to the original soil pH of 7.7 after 1 month. This restabilization at the original soil pH was not anticipated. Soil 1 was still considered a separate soil in the statistical analysis.

The pH of medium treated with $\text{Ca}(\text{OH})_2$ (Soil 3) was increased from 7.7 to 8.3 and maintained at that level throughout the experiment. Spinach seedlings were severely wilted soon after transplanting and a large number of plants died. Those that did survive grew very slowly. Spinach receiving N applications were the most severely affected indicating the problem may have been due to high salts. Plant growth was so severely affected in Soil 3 that the data could not be used.

Soil NH_4 -N, NO_3 -N and pH

Soil NH_4 -N concentrations measured at harvest were highest from NH_4 -nitrapyrin applications and increased with increasing N application rates (Table 11) while soil NO_3 -N concentrations increased with N and decreased with NH_4 -nitrapyrin applications (Table 12). Nitrapyrin applied to $(\text{NH}_4)_2\text{SO}_4$ at 1.5 ppm (dry soil weight basis) was very effective in preventing the oxidation of NH_4 to NO_3 for 88 days, the length of the experiment. A significant

Table 11. Mean soil $\text{NH}_4\text{-N}$ concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> rate ppm	0	Soil 1	4	7	61	24	24
		Soil 2	3	9	59	24	
	1.5	Soil 1	4	74	174	84	79
		Soil 2	5	43	176	75	
Soil X Nitrogen	Soil 1	4	41	118			
	Soil 2	4	26	118			
Nitrogen		4	33	118			

Analysis of variance

Source	df	F values
Block	4	2.40
Soil	1	4.02
Nitrogen	2	854.55 **
Nitrapyrin	1	563.23 **
Soil X Nitrogen	2	4.12 *
Soil X Nitrapyrin	1	3.82
Nitrogen X Nitrapyrin	2	199.90 **
Soil X Nitrogen X Nitrapyrin	2	6.64 **
Error Mean Square	44	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid

^Y Untreated soil

Table 12. Mean soil NO₃-N concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> <u>rate</u> ppm	0	Soil 1	3	33	151	62	50
		Soil 2	3	26	87	39	
	1.5	Soil 1	3	4	4	4	3
		Soil 2	3	4	3	3	
Soil X Nitrogen	Soil 1	3	19	73			
	Soil 2	3	15	46			
Nitrogen		3	17	61			

Analysis of variance

Source	df	F values
Block	4	1.60
Soil	1	5.85 *
Nitrogen	2	51.87 **
Nitrapyrin	1	91.41 **
Soil X Nitrogen	2	4.36 *
Soil X Nitrapyrin	1	5.60 *
Nitrogen X Nitrapyrin	2	51.45 **
Soil X Nitrogen X Nitrapyrin	2	4.24 *
Error Mean Square	44	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid

^Y Untreated soil

nitrogen X nitrapyrin interaction was anticipated with no soil NH_4 -N or NO_3 -N concentration differences between no N control treatments with and without nitrapyrin. Significant soil system interactions resulted from higher NH_4 -N and NO_3 -N concentrations from both N application rates in Soil 1.

The original pH of the untreated soil (Soil 2) was 7.7 and the pH of soil treated with acetic acid (Soil 1) was also 7.7 1 month after treatment. At harvest, the pH of soil with no N with and without nitrapyrin was 7.5 from both Soil 1 and Soil 2. The pH of soils with 400 and 800 mg N/2 kg soil without nitrapyrin of both Soil 1 and Soil 2 was 5.8 and 5.2 respectively. The pH of soil with 400 and 800 mg N/2 kg soil with 1.5 ppm nitrapyrin of both Soil 1 and Soil 2 was 6.7 and 6.5 respectively. Apparently the nitrification activity from N applications without nitrapyrin was more acidifying than NH_4 nutrition from NH_4 -nitrapyrin applications. The soils in this experiment had poor buffering capacity.

Spinach Yield

Observations throughout the experiment indicated no visual plant size differences between N treatments with and without nitrapyrin for approximately 2/3 of the experiment. Spinach plants from N treatments with nitrapyrin were substantially smaller at harvest (Table 13). The low N application rate produced larger plants than the high N rate. There was a slightly larger plant size difference

Table 13. Mean spinach yields (g/plant) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> <u>rate</u> ppm	0	Soil 1	4.5	34.3	25.6	21.5	20.6
		Soil 2	4.1	29.2	25.8	19.7	
	1.5	Soil 1	5.4	17.8	17.5	13.6	14.2
		Soil 2	4.9	18.6	21.0	14.8	
Soil X Nitrogen	Soil 1	5.0	26.0	21.6			
	Soil 2	4.5	23.9	23.4			
Nitrogen		4.7	25.0	22.5			

Analysis of variance

Source	df	F values
Block	14	.51
Soil	1	.37
Nitrogen	2	1088.89 **
Nitrapyrin	1	374.32 **
Soil X Nitrogen	2	8.91 **
Soil X Nitrapyrin	1	15.25 **
Nitrogen X Nitrapyrin	2	116.50 **
Soil X Nitrogen X Nitrapyrin	2	5.09 **
Error Mean Square	334	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid
^Y Untreated soil

between low N applications with and without nitrapyrin in Soil 1 which resulted in significant soil interactions.

Spinach grown with NH_4 -nitrapyrin applications exhibited NH_4 toxicity symptoms of stunted plant size (74), poor root development (12, 63, 74) and marginal leaf burn (5, 74) at harvest. Plants from NH_4 -nitrapyrin treatments began to wilt sooner than those plants from N applications without nitrapyrin when water stress conditions prevailed (12, 101).

Leaf Color

Spinach from N and nitrapyrin treatments began exhibiting visual leaf color differences halfway through the experiment. Tissue from no N control treatments were visibly chlorotic. Visual leaf color differences were most evident at harvest. Ammonium-nitrapyrin applications produced spinach with visually darker green tissue. This is supported in Table 14 by leaf light absorption measurements. There was no leaf color difference between no N control treatments with and without nitrapyrin which resulted in a significant nitrogen X nitrapyrin interaction. Plants grown in Soil 2 exhibited darker green leaf color than plants grown in Soil 1.

Leaf P, Fe and Zn Concentrations

Ammonium-nitrapyrin treatments produced spinach with higher leaf concentrations of P but not Fe and Zn (Tables 15, 16 and 17),

Table 14. Mean leaf light absorption values (green color) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> <u>rate</u> ppm	0	Soil 1	.68	.80	.82	.77	.78
		Soil 2	.71	.84	.82	.79	
	1.5	Soil 1	.74	.89	.92	.85	.86
		Soil 2	.74	.94	.92	.87	
Soil X Nitrogen	Soil 1	.71	.85	.87			
	Soil 2	.73	.89	.87			
Nitrogen		.72	.87	.87			

Analysis of variance

Source	df	F values
Soil	1	8.30 **
Nitrogen	2	213.08 **
Nitrapyrin	1	144.64 **
Soil X Nitrogen	2	3.08 *
Soil X Nitrapyrin	1	.02
Nitrogen X Nitrapyrin	2	5.66 **
Soil X Nitrogen X Nitrapyrin	2	1.10
Error Mean Square	708	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid
^Y Untreated soil

Table 15. Mean leaf P concentrations (%) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> <u>rate</u> ppm	0	Soil 1	1.67	1.57	1.38	1.54	1.50
		Soil 2	1.64	1.41	1.32	1.46	
	1.5	Soil 1	1.66	1.58	1.43	1.56	1.54
		Soil 2	1.60	1.52	1.45	1.52	
Soil X Nitrogen		Soil 1	1.66	1.57	1.41		
		Soil 2	1.62	1.46	1.39		
Nitrogen			1.64	1.52	1.40		

Analysis of variance

Source	df	F values
Block	4	2.38
Soil	1	56.75 **
Nitrogen	2	378.25 **
Nitrapyrin	1	32.50 **
Soil X Nitrogen	2	13.75 **
Soil X Nitrapyrin	1	13.00 **
Nitrogen X Nitrapyrin	2	19.75 **
Soil X Nitrogen X Nitrapyrin	2	6.88 **
Error Mean Square	44	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid

^Y Untreated soil

Table 16. Mean leaf Fe concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> <u>rate</u> ppm	0	Soil 1	494	330	232	352	315
		Soil 2	402	280	152	278	
	1.5	Soil 1	450	384	294	376	344
		Soil 2	354	300	294	312	
Soil X Nitrogen	Soil 1	477	357	258			
	Soil 2	378	290	223			
Nitrogen		425	324	240			

Analysis of variance

Source	df	F values
Block	4	3.70 *
Soil	1	20.86 **
Nitrogen	2	50.15 **
Nitrapyrin	1	3.69
Soil X Nitrogen	2	.85
Soil X Nitrapyrin	1	.11
Nitrogen X Nitrapyrin	2	7.43 **
Soil X Nitrogen X Nitrapyrin	2	1.00
Error Mean Square	44	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid

^Y Untreated soil

Table 17. Mean leaf Zn concentrations (ppm) at harvest for the main and combined effect of N and nitrapyrin applications for Soil 1^Z and Soil 2^Y. Spinach greenhouse experiment 1974-1975.

		<u>Nitrogen rate</u> mg/2 kg medium			Soil X		
		0	400	800	Nitrapyrin	Nitrapyrin	
<u>Nitrapyrin</u> <u>rate</u> ppm	0	Soil 1	250	148	116	171	147
		Soil 2	220	91	57	123	
	1.5	Soil 1	224	154	150	176	155
		Soil 2	176	110	117	134	
Soil X Nitrogen	Soil 1	237	151	133			
	Soil 2	198	101	87			
Nitrogen		217	126	110			

Analysis of variance

Source	df	F values
Block	4	.34
Soil	1	42.08 **
Nitrogen	2	92.38 **
Nitrapyrin	1	1.30
Soil X Nitrogen	2	.23
Soil X Nitrapyrin	1	.22
Nitrogen X Nitrapyrin	2	11.43 **
Soil X Nitrogen X Nitrapyrin	2	.84
Error Mean Square	44	

* 5% significance level

** 1% significance level

^Z Soil treated with acetic acid

^Y Untreated soil

however, increased Fe and Zn concentrations from NH_4 -nitrapyrin applications were observed. The highly significant nitrogen X nitrapyrin interactions with P, Fe and Zn concentrations resulted from reduced leaf concentrations of these elements from no N control treatments with nitrapyrin while NH_4 -nitrapyrin treatments had higher leaf P, Fe and Zn concentrations. Spinach P, Fe and Zn concentrations were unquestionably higher from no N control treatments and lowest from high N applications. Leaf concentrations of P, Fe and Zn were also greater from Soil 1 than Soil 2. The leaf concentrations of P and Zn were extremely high in this experiment. The reason for the high values could not be determined.

Field Experiment - 1975

Soil NH_4 -N, NO_3 -N and pH

Nitrapyrin effectively suppressed nitrification as indicated by higher soil NH_4 -N (Table 18) and lower soil NO_3 -N (Table 19) concentrations resulting from N applications with nitrapyrin. The 2 highest nitrapyrin application rates produced higher soil NH_4 -N concentrations than the low rate. Soil NH_4 -N and NO_3 -N were measured from each plot 18, 28, 42, 48 and 56 days from planting. Response surfaces in Appendix Figures 1 to 6 illustrate NH_4 -N and NO_3 -N concentration differences between nitrapyrin application rates. Soil samples taken 18 days from planting were combined rather than

Table 18. Mean soil $\text{NH}_4\text{-N}$ concentrations (ppm) for the main and combined effect of N and nitrapyrin applications 28, 42, 48 and 56 days from planting. Spinach field experiment - 1975.

	Time	<u>Nitrogen rate</u> kg/ha			Time X Nitrapyrin	Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		0	45	90			
0	28	14	14	16	15		
	42	11	12	11	11		
	48	9	12	10	10	11	11
	56	9	9	9	9		
140	28	13	42	68	41		
	42	10	27	44	27		
	48	12	21	32	22	26	
	56	9	11	17	12		
<u>Nitrapyrin</u> rate g/ha	28	13	58	101	57		
	42	9	32	88	43		
	48	11	23	48	27	35	33
	56	9	12	18	13		
280	28	11	86	90	62		
	42	10	38	69	39		
	48	10	23	44	26	37	
	56	9	16	37	21		
420	28	11	86	90	62		
	42	10	38	69	39		
	48	10	23	44	26	37	
	56	9	16	37	21		
Time X Nitrogen	28	13	50	69			
	42	10	27	53			
	48	11	20	34			
	56	9	12	20			
Nitrogen X Nitrapyrin	0	11	12	12			
	140	11	25	40			
	280	11	31	64			
	420	10	41	60			
Nitrogen		11	27	44			

Table 18, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	5	2.14
Sample Times	3	55.80 **
Nitrogen	2	120.07 **
Nitrapyrin	3	45.79 **
No Nitrapyrin vs. Nitrapyrin	1	111.70 **
140 vs. 280 g/ha	1	15.57 **
280 vs. 420 g/ha	1	.71
Time X Nitrogen	6	12.02 **
Time X Nitrapyrin	9	5.56 **
Nitrogen X Nitrapyrin	6	16.51 **
Time X Nitrogen X Nitrapyrin	18	2.46 **
Error Mean Square	235	

* 5% significance level

** 1% significance level

Table 19. Mean soil NO₃-N concentrations (ppm) for the main and combined effect of N and nitrapyrin applications 28, 42, 48 and 56 days from planting. Spinach field experiment - 1975.

	Time	<u>Nitrogen rate</u> kg/ha			Time X		No Nitrapyrin vs. Nitrapyrin
		0	45	90	Nitrapyrin	Nitrapyrin	
0	28	20	31	45	32		
	42	8	13	12	11		
	48	6	9	9	9	15	15
	56	7	7	7	7		
140	28	19	21	20	20		
	42	7	9	12	9		
	48	6	10	16	11	12	
	56	5	8	14	9		
<u>Nitrapyrin</u> <u>rate</u> g/ha	28	15	14	15	15		
	42	7	11	8	9		
	280	48	6	9	10	8	10
	56	9	7	9	8		11
420	28	14	12	21	16		
	42	7	10	14	10		
	48	5	8	13	9	11	
	56	7	7	17	10		
Time X Nitrogen	28	17	20	25			
	42	7	11	12			
	48	6	9	13			
	56	7	7	12			
Nitrogen X Nitrapyrin	0	10	15	19			
	140	9	12	16			
	280	9	10	11			
	420	8	9	16			
Nitrogen		9	12	15			

Table 19, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	5	.52
Sample Time	3	30.20 **
Nitrogen	2	17.86 **
Nitrapyrin	3	3.83 *
No Nitrapyrin vs. Nitrapyrin	1	9.14 **
140 vs. 280 g/ha	1	2.36
280 vs. 420 g/ha	1	.57
Time X Nitrogen	6	.39
Time X Nitrapyrin	9	3.86 **
Nitrogen X Nitrapyrin	6	1.56
Time X Nitrogen X Nitrapyrin	18	.99
Error Mean Square	235	

* 5% significance level

** 1% significance level

divided into replications and treatment means were reported in Appendix Figures 1 to 6 but were not included in the statistical analysis in Tables 18 and 19. Nitrapyrin increased soil $\text{NH}_4\text{-N}$ only with N applications which resulted in a significant nitrogen X nitrapyrin interaction. Greatest $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ concentration differences between N applications with and without nitrapyrin were 18 days from planting with declining concentrations and concentration differences with time which resulted in the significant time X nitrapyrin interactions. Although $\text{NH}_4\text{-N}$ concentrations were declining with time, N applications with nitrapyrin had higher soil $\text{NH}_4\text{-N}$ concentrations than N applications without nitrapyrin 56 days from planting.

The original soil pH was 7.9 and throughout the experiment the soil pH from all treatments was maintained between 7.9 and 8.1 with no pH differences between treatments. This soil had a strong buffering capacity.

Spinach Yield

Nitrapyrin reduced spinach yields 28 days from planting. There were no yield differences 42 days from planting and relatively small yield increases with nitrapyrin 48 days from planting. Nitrapyrin increased spinach yields substantially 56 days from planting, enough to significantly increase overall yields which involved the combination of all 4 sample times (Table 20). Response surfaces in Appendix Figures 7 to 9 illustrate yield increases with nitrapyrin.

Table 20. Mean spinach yields (MT/ha) for the main and combined effect of N and nitrapyrin applications 28, 42, 48 and 56 days from planting. Spinach field experiment - 1975.

	Time	<u>Nitrogen rate</u> kg/ha			Time X Nitrapyrin	Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		0	45	90			
0	28	0.8	1.7	1.6	1.4		
	42	3.0	5.3	7.0	5.1		
	48	6.3	8.7	12.1	9.0	6.6	6.6
	56	7.9	9.9	14.3	10.7		
140	28	0.9	1.6	1.6	1.4		
	42	3.5	6.4	7.5	5.8		
	48	6.2	9.4	15.1	10.2	7.6	
	56	7.4	13.3	17.8	12.8		
<u>Nitrapyrin</u> <u>rate</u> g/ha	28	0.9	1.3	1.2	1.1		
	42	2.9	5.9	6.1	5.0		
	280	5.5	10.8	13.0	9.8	7.2	7.4
	56	7.6	14.1	16.9	12.9		
420	28	0.8	1.2	1.2	1.1		
	42	2.7	6.7	7.0	5.5		
	48	5.6	9.7	14.2	9.8	7.5	
	56	7.6	13.7	19.3	13.5		
Time X Nitrogen	28	0.9	1.5	1.4			
	42	3.0	6.1	6.9			
	48	5.9	9.7	13.6			
	56	7.6	12.8	17.1			
Nitrogen X Nitrapyrin	0	4.5	6.4	8.8			
	140	4.5	7.7	10.5			
	280	4.2	8.0	9.3			
	420	4.2	7.8	10.4			
Nitrogen		4.4	7.5	9.7			

Table 20, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	5	12.48 **
Sample Time	3	400.19 **
Nitrogen	2	189.56 **
Nitrapyrin	3	3.87 *
No Nitrapyrin vs. Nitrapyrin	1	7.90 **
140 vs. 280 g/ha	1	3.57
280 vs. 420 g/ha	1	.40
Time X Nitrogen	6	22.46 **
Time X Nitrapyrin	9	1.89
Nitrogen X Nitrapyrin	6	1.47
Time X Nitrogen X Nitrapyrin	18	.86
Error Mean Square	235	

* 5% significance level

** 1% significance level

The cross-hatch at the top of each response surface represents expected spinach yields 4 days beyond the last measured harvest. This was done to show an even greater anticipated yield response to nitrapyrin with time. Anticipated yields 60 days from planting were determined by a visual extrapolation. There were no yield differences among nitrapyrin application rates. Nitrapyrin increased yields only with NH_4 applications.

Spinach plots were very uniform and the high N application (90 kg/ha) did not reduce stand. Yields were greatest from the high N application. Spinach was exhibiting N deficiency symptoms from the no N control treatments at the first sample period.

Leaf Color

Visual color differences were observed 18 days from planting with the no N control treatments exhibiting chlorotic leaves. Spinach leaf color was visually darker green from N applications with nitrapyrin from the second sample period, 42 days from planting, through the final harvest. Appendix Figure 10 shows color differences between 90 kg N/ha with and without nitrapyrin. Darker green leaf color observed with nitrapyrin was supported by leaf light absorption treatment means in Table 21 and Appendix Figures 11 to 13. The 2 highest nitrapyrin applications produced tissue with darker green leaf color than the low nitrapyrin application. Nitrapyrin increased green leaf color only with N applications which resulted in a

Table 21. Mean leaf light absorption values (green color) for the main and combined effect of N and nitrapyrin applications 42, 48 and 56 days from planting. Spinach field experiment - 1975.

		<u>Nitrogen rate</u> kg/ha			Time X		No Nitrapyrin vs. Nitrapyrin	
		0	45	90	Nitrapyrin	Nitrapyrin		
<u>Time</u>								
<u>Nitrapyrin</u> <u>rate</u> g/ha	0	42	.74	.74	.76	.75		
		48	.74	.74	.74	.74	.74	.74
		56	.72	.74	.75	.74		
	140	42	.75	.79	.77	.77		
		48	.73	.77	.78	.76	.76	
		56	.72	.77	.80	.76		
	280	42	.76	.78	.80	.78		
		48	.73	.78	.80	.77	.77	.77
		56	.73	.78	.79	.77		
420	42	.76	.81	.80	.79			
	48	.73	.79	.79	.78	.78		
	56	.73	.78	.81	.77			
Time X Nitrogen	42	.75	.78	.78				
	48	.73	.77	.78				
	56	.72	.77	.79				
Nitrogen X Nitrapyrin	0	.73	.74	.75				
	140	.73	.78	.78				
	280	.74	.78	.80				
	420	.74	.80	.80				
Nitrogen		.74	.77	.78				

Table 21, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	5	1.64
Sample Time	2	6.76 **
Nitrogen	2	103.32 **
Nitrapyrin	3	32.28 **
No Nitrapyrin vs. Nitrapyrin	1	84.60 **
140 vs. 280 g/ha	1	4.24 *
280 vs. 420 g/ha	1	1.84
Time X Nitrogen	4	3.48 **
Time X Nitrapyrin	6	.40
Nitrogen X Nitrapyrin	6	5.96 **
Time X Nitrogen X Nitrapyrin	12	1.28
Error Mean Square	1260	

* 5% significance level

** 1% significance level

significant nitrogen X nitrapyrin interaction. Leaf light absorption values decreased with time from the no N control and low N application rate but increased slightly with time from the high N application rate.

Leaf P, Fe and Zn Concentrations

Nitrapyrin applications increased leaf P, Fe and Zn concentrations (Tables 22, 23 and 24, Appendix Figures 14 to 22). The response surfaces in the appendix figures indicate fairly consistent P and Fe concentration increases with increasing nitrapyrin rates. Matured leaves from nitrapyrin application rates of 280 and 420 g/ha had higher P and Fe concentrations than leaves from the nitrapyrin rate of 140 g/ha. There were no P, Fe or Zn concentration differences between the 2 highest nitrapyrin rates. The highest P concentration increases with nitrapyrin were 28 days from planting. The lack of nitrogen X nitrapyrin interactions with P, Fe and Zn concentrations indicated that the effect of nitrapyrin applications on leaf concentrations of these elements was the same with and without N applications. Phosphorous, Fe and Zn concentrations were increased with nitrapyrin in the absence of an NH_4 application.

Leaf P and Zn concentrations were highest from no N control treatments and lowest from high N applications. Although Zn concentrations were significantly decreased with increasing N applications, relative differences were small, Leaf Fe concentration

Table 22. Mean leaf P concentrations (%) for the main and combined effect of N and nitrapyrin applications 28, 42, 48 and 56 days from planting. Spinach field experiment - 1975.

	Time	Nitrogen rate kg/ha			Time X		No Nitrapyrin vs. Nitrapyrin
		0	45	90	Nitrapyrin	Nitrapyrin	
0	28	.74	.68	.68	.70		
	42	.73	.71	.67	.70		
	48	.63	.62	.60	.63	.67	.67
	56	.65	.64	.63	.64		
140	28	.75	.81	.77	.79		
	42	.79	.71	.68	.73		
	48	.67	.63	.61	.64	.70	
	56	.72	.61	.63	.65		
<u>Nitrapyrin</u> rate g/ha	28	.79	.81	.77	.79		
	42	.85	.74	.71	.77		
	280	.70	.66	.63	.66	.72	.72
	56	.72	.65	.65	.67		
420	28	.86	.81	.78	.82		
	42	.83	.75	.72	.77		
	48	.69	.66	.65	.67	.74	
	56	.76	.67	.65	.69		
Time X Nitrogen	28	.79	.78	.75			
	42	.80	.73	.70			
	48	.68	.64	.62			
	56	.71	.65	.64			
Nitrogen X Nitrapyrin	0	.69	.67	.65			
	140	.73	.69	.67			
	280	.77	.72	.69			
	420	.79	.72	.70			
Nitrogen		.75	.70	.68			

Table 22, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	4	3.39 *
Sample Time	3	64.67 **
Nitrogen	2	28.48 **
Nitrapyrin	3	17.91 **
No Nitrapyrin vs. Nitrapyrin	1	40.21 **
140 vs. 280 g/ha	1	6.06 *
280 vs. 420 g/ha	1	1.33
Time X Nitrogen	6	2.09
Time X Nitrapyrin	9	.97
Nitrogen X Nitrapyrin	6	.55
Time X Nitrogen X Nitrapyrin	18	.97
Error Mean Square	188	

* 5% significance level

** 1% significance level

Table 23. Mean leaf Fe concentrations (ppm) for the main and combined effect of N and nitrapyrin applications 28, 42, 48 and 56 days from planting. Spinach field experiment - 1975.

	Time	Nitrogen rate kg/ha			Time X Nitrapyrin	Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		0	45	90			
0	28	673	610	602	628		
	42	452	448	450	450		
	48	512	560	420	497	497	497
	56	420	418	395	411		
140	28	588	608	638	611		
	42	503	478	450	477		
	48	498	550	485	511	508	
	56	422	445	426	431		
<u>Nitrapyrin</u> <u>rate</u> g/ha	28	724	628	625	659		
	42	590	524	478	531		
	280	542	570	530	547	551	535
	56	434	480	484	466		
420	28	588	674	650	637		
	42	530	652	482	555		
	48	528	585	506	540	547	
	56	444	455	466	455		
Time X Nitrogen	28	643	630	629			
	42	519	526	465			
	48	520	566	524			
	56	430	450	441			
Nitrogen X Nitrapyrin	0	514	509	467			
	140	503	520	500			
	280	573	551	529			
	420	523	592	526			
Nitrogen		528	543	506			

Table 23, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	4	3.48 **
Sample Time	3	27.89 **
Nitrogen	2	2.93
Nitrapyrin	3	4.05 **
No Nitrapyrin vs. Nitrapyrin	1	6.06 *
140 vs. 280 g/ha	1	4.77 *
280 vs. 420 g/ha	1	.01
Time X Nitrogen	6	1.16
Time X Nitrapyrin	9	.61
Nitrogen X Nitrapyrin	6	.36
Time X Nitrogen X Nitrapyrin	18	.52
Error Mean Square	188	

* 5% significance level

** 1% significance level

Table 24. Mean leaf Zn concentrations (ppm) for the main and combined effect of N and nitrapyrin applications 28, 42, 48 and 56 days from planting. Spinach field experiment - 1975.

	Time	<u>Nitrogen rate</u> kg/ha			Time X Nitrapyrin	Nitrapyrin	No Nitrapyrin vs. Nitrapyrin
		0	45	90			
0	28	42	50	55	49		
	42	62	62	59	61		
	48	61	60	55	59	56	56
	56	53	57	54	55		
140	28	47	53	56	52		
	42	70	61	62	64		
	48	62	58	56	59	59	
	56	62	57	58	59		
<u>Nitrapyrin</u> <u>rate</u> g/ha	28	53	53	53	53		
	42	75	66	61	67		
	280	48	65	58	60	60	60
	56	63	58	56	59		
420	28	53	53	53	53		
	42	72	66	60	66		
	48	61	67	62	63	60	
	56	62	57	59	59		
Time X Nitrogen	28	49	52	54			
	42	70	64	61			
	48	62	61	58			
	56	60	57	57			
Nitrogen X Nitrapyrin	0	55	57	56			
	140	60	57	58			
	280	64	59	57			
	420	62	61	59			
Nitrogen		60	59	58			

Table 24, cont'd.

<u>Analysis of variance</u>		
Source	df	F values
Block	4	3.93 **
Sample Time	3	33.58 **
Nitrogen	2	3.18 *
Nitrapyrin	3	4.73 **
No Nitrapyrin vs. Nitrapyrin	1	11.50 **
140 vs. 280 g/ha	1	1.71
280 vs. 420 g/ha	1	.04
Time X Nitrogen	6	3.77 **
Time X Nitrapyrin	9	.55
Nitrogen X Nitrapyrin	6	1.77
Time X Nitrogen X Nitrapyrin	18	.58
Error Mean Square	188	

* 5% significance level

** 1% significance level

differences among N applications were not significant, however, the low N application produced the highest leaf Fe concentrations.

Tissue P and Fe concentrations were at a measured maximum 28 days from planting with decreasing concentrations with time.

Tissue Zn concentrations were at a measured maximum 42 days from planting with concentrations decreasing with time.

DISCUSSION AND CONCLUSIONS

Spinach Yields

Nitrapyrin with $(\text{NH}_4)_2\text{SO}_4$ applications increased spinach yields by influencing soil N forms in the 1974 field experiment. Nitrapyrin with $(\text{NH}_4)_2\text{SO}_4$ increased yield in the 1975 field experiment by increased soil N retention. The greenhouse experiment indicated that NH_4 -nitrapyrin applications can reduce plant growth by prolonged nitrification suppression when high NH_4 application rates are employed.

Soil N measurements from the 1974 field experiment at harvest indicated that N applications without nitrapyrin resulted in low levels of NH_4 and high levels of NO_3 . Low NH_4 was expected because nitrification activity was not suppressed, however, high NO_3 was not anticipated. Nitrate resulting from early nitrification should have leached from the soil system. The spinach plots apparently did not receive enough water to leach the mobile NO_3 form from the soil.

Increased yields with NH_4 -nitrapyrin applications in the 1974 field experiment appear to be due to N forms rather than increased soil N retention. Yields were lowest from N applications without nitrapyrin which resulted in NO_3 as the predominant N form at harvest. Highest yields were produced from N applications with the low

nitrapyrin rate resulting in both NO_3 and NH_4 available to the plant at harvest. This is in agreement with a number of other investigations that found maximum plant growth when NH_4 was available in combination with NO_3 (22, 77, 83, 127, 135, 140).

Soil N measurements from the 1975 field experiment were characteristic of a highly leached soil system. Soil concentrations of NO_3 were very low at all sample times as compared to concentrations of less mobile NH_4 . Soil samples from the final 3 sample times were taken 2 to 3 days following irrigation which may further explain extremely low NO_3 measurements.

Increases in final spinach yields from N applications with nitrapyrin in the 1975 field experiment were attributed to greater soil N retention (50, 58, 85, 114, 123). Measuring yield differences between N applications with and without nitrapyrin at various growth stages gave a more accurate indication when nitrification suppression was affecting spinach yields. Yields from N applications without nitrapyrin were higher 28 days from planting, most probably due to higher N availability. Ammonium from NH_4 -nitrapyrin applications had not moved an appreciable distance from the band to become available to the plant. Higher soil concentrations of the more mobile NO_3 from N applications without nitrapyrin resulted in better spinach growth at this time. With the exception of this first sample time, there were no yield differences between N applications with and without

nitrapyrin until the final measured harvest, 56 days from planting, when yields from N applications with nitrapyrin were substantially higher and were continuing to increase. Yield benefits from nitrapyrin were greatest in this experiment when harvest was delayed until 56 days from planting. It appears that increased soil N retention from NH_4 -nitrapyrin applications is the reason for increased yields, but because the 1974 field experiment indicated yield responses due to N form and combinations of N forms, the possibility of this being at least a partial reason for increased yields in the 1975 field experiment cannot be dismissed. Nitrapyrin increased the efficiency of N applications in the 1974 and 1975 field experiments. Spinach yields from the low N application with nitrapyrin in 1975 were equivalent to yields produced from twice as much N without nitrapyrin.

One and one half ppm nitrapyrin completely suppressed nitrification for the length of the experiment, 88 days, in the greenhouse. This nitrapyrin application rate was considerably higher than the maximum field experiment rate of 0.25 ppm. In the greenhouse experiment, spinach was sustained almost exclusively by NO_3 in the case of N applications without nitrapyrin and NH_4 from N applications with nitrapyrin. Spinach appeared to grow equally as well with either N form for the first 2/3 of growth, however, continued concentrated NH_4 from NH_4 -nitrapyrin applications greatly reduced spinach growth during the final 1/3 or exponential phase of growth. These results are in

complete agreement with results by Spratt and Gasser (113) which indicate the leafy vegetable kale to show no preference for either N form early but a distinct preference for NO_3 during later growth stages.

In 1967, B. L. Bengtsson (11) grew spinach with the nitrification suppressant, nitrapyrin. Ammonium sulfate was broadcast at N rates of 80 and 160 kg/ha with and without nitrapyrin. Nitrapyrin was applied at a rate of 2% of the N applied. Bengtsson reported N applications with nitrapyrin produced lower yields than the same N applications without nitrapyrin. Bengtsson suspected very little leaching in his experiment and attributed smaller plants resulting from concentrated NH_4 from prolonged nitrification suppression as the reason for reduced yields. Decreased plant size with NH_4 -nitrapyrin applications in the greenhouse are in agreement with Bengtsson's conclusions, however, increased yields from concentrated NH_4 conditions from N applications with the high nitrapyrin rate in the 1974 field experiment contradict Bengtsson's results.

Ammonium may be an adequate N form for plant growth if alkaline pH is maintained (5, 6, 7, 74, 108, 134, 139) and K is not deficient (4, 22, 71, 129, 139). The soil in the 1974 field experiment had a high pH, lime was high and bulk soil and possibly rhizosphere pH was buffered and K concentration was very high. The soil in the 1974 field experiment supported good plant growth with NH_4 nutrition. The

initial pH of the greenhouse soil was high and the level of available K was high but the absence of lime allowed for an unbuffered condition and resulted in a large decrease in pH by harvest. The greenhouse soil-perlite medium did not support plant growth with NH_4 nutrition. Stunted plant growth from N applications with nitrapyrin in the greenhouse agrees with reduced plant growth observed from other unbuffered or poorly buffered NH_4 solution experiments (5, 15, 62, 63, 98). Spinach growth from N applications with nitrapyrin in the greenhouse exhibited NH_4 toxicity symptoms. Bengtsson did not describe the soil in his experiment but from his other treatments it did not seem to be alkaline.

As was mentioned earlier, many past NH_4 nutrition studies have employed solution experiments because of the inability to maintain NH_4 in a soil system due to nitrification. All 3 experiments indicate that NH_4 versus NO_3 nutrition studies can be done in soil with nitrapyrin to suppress nitrification, thus removing many of the difficulties and criticism involved with solution experiments.

The high N applications in the 1974 experiment severely burned spinach seedlings with the problem somewhat less when nitrapyrin was applied. The influence that nitrapyrin had on lessening the degree of fertilizer burn was most probably indirect. High concentrations of mobile NO_3 resulting from nitrification from N applications without nitrapyrin may have caused the most severe fertilizer burn. Lower

N rates used in the 1975 field experiment and a deeper band prevented severe fertilizer burn. Spinach yields in 1975 were much higher than 1974 because of 40% higher plant populations.

Nitrapyrin rates in the 1975 field experiment were lower than rates in 1974 because N applications with the high rate of nitrapyrin seemed to limit plant growth by suppressing nitrification through the entire experiment. The minimum concentration of nitrapyrin required to effectively suppress nitrification was not determined in this study. Nitrification was effectively suppressed and yields increased from N applications with a nitrapyrin rate as low as 140 g/ha in the 1975 field experiment. Higher nitrapyrin applications did suppress nitrification for longer times and the 140 g/ha rate was probably approaching the minimum effective threshold. Future investigations with nitrapyrin should include logarithmic applications to determine effective application rates for economic considerations.

Some plants have indicated a strong preference for NH_4 (21, 34, 40, 54, 99, 109, 126), others prefer NH_4 during early growth stages (45, 47, 76, 78, 87, 106, 113), and many have some degree of tolerance for NH_4 (2, 3, 18, 29, 70, 112, 113, 138). Nitrapyrin may be a tool in controlling the form of N supplied to plants. Nitrapyrin could increase yields of plants that have some preference or tolerance for NH_4 by suppressing nitrification for the length of time that NH_4 is preferred or tolerated. This study indicated spinach to have a

tolerance to NH_4 for the first 2/3 of growth and yields were increased in the field experiments by low nitrapyrin application rates. The greenhouse experiment indicated that excessive nitrapyrin can severely reduce plant growth by suppression of nitrification for the entire growth period. Concentrated NH_4 has been shown to produce good plant growth when N is limiting (22, 45, 106, 131, 133). Further investigations with nitrapyrin should include high nitrapyrin application rates with low N rates.

The activity of nitrapyrin is highly dependent on soil characteristics and environmental conditions (36). Control of the form of N supplied to the plant at various growth stages with nitrapyrin is very difficult and excessive nitrapyrin applications could result in severe crop losses from concentrated NH_4 conditions. By applying a relatively high rate of nitrapyrin with low N at planting and side-dressing NO_3 as the crop reached the critical stage when NH_4 alone could no longer be tolerated, the rate of nitrapyrin applied would be much less critical. This combination of N forms should enhance growth as was indicated in the 1974 field experiment. Results from a recently completed greenhouse experiment not reported here indicated maximum spinach yields when NH_4 supplemented NO_3 nutrition. This method of nitrapyrin application may increase the efficiency of N applications, increase crop yields and prevent possible crop reductions due to a preponderance of NH_4 in the root zone. Nitrapyrin may be used to

in effect "prescription feed" a crop once its relative preference for NH_4 and NO_3 is known.

For future nitrapyrin studies, priorities should be placed on important crops that have indicated possible preference or tolerance to NH_4 such as onion (74, 138) and maize (2). The tolerance of maize to concentrated NH_4 has been attributed to the C-4 pathway. Other crops with this metabolic characteristic may also benefit from nitrapyrin. Research should also begin with breeding field crops that might be more tolerant to or prefer NH_4 . If future crop varieties could be developed to include the ability to utilize concentrated NH_4 throughout the entire growing season, increases in N application efficiency with nitrapyrin would be incalculable.

Leaf Color

Spinach leaf color was visually a darker green when produced from N applications with nitrapyrin in all 3 experiments. Darker green color with nitrapyrin was not evident during early growth stages but only when spinach was in the exponential phase of growth. Leaf light absorption measurements from harvest in the 1974 and 1975 field experiments and greenhouse experiment, as well as measurements taken 14 to 8 days prior to the final harvest in the 1975 field experiment, indicate darker green leaf color from N applications with nitrapyrin.

The 1974 field experiment showed lower significance of treatment leaf color differences than expected. The greenhouse and 1975 field experiments indicated that a large number of leaf measurements are required to indicate highly significant leaf color differences between N applications with and without nitrapyrin. Light absorption measurements on savoy spinach leaves were difficult and readings were extremely variable. The leaf light absorption measurements used for statistical analysis in the 1974 field experiment were the average of 10 rather than individual measurements and significant differences were not as large as expected.

Increases in leaf Fe concentration generally accompanied increases in green leaf color in this study. Machold (69) attributed darker green leaf color with NH_4 nutrition, resulting from nitrification suppression, to increased Fe utilization rather than increased Fe absorption. The correlation between tissue Fe concentration and spinach leaf color was not an objective of this study, but comparisons not reported here indicated that leaf Fe concentrations did not correlate well with green leaf color.

Little work has been done to correlate the degree of green tissue color to nutritional quality, particularly pro-vitamin A content (8, 125). Spinach tissue from this study and future experiments will be used to determine if the pro-vitamin A content is linked to green tissue color. A positive correlation would indicate that

nitrification suppression can be used to increase the nutritional quality, as well as the market quality, of spinach.

Leaf P, Fe and Zn Concentrations

Ammonium-nitrapyrin applications consistently increased leaf P and Fe concentrations in all 3 experiments, while leaf Zn concentrations indicated an increase from NH_4 -nitrapyrin applications in the greenhouse and slight increases in the 1975 field experiment.

Higher leaf P and Fe concentrations from NH_4 -nitrapyrin treatments in the 1974 field experiment can only partially be attributed to NH_4 because treatments that consistently produced leaves with the highest P and Fe concentrations at harvest also produced the greatest yields. Maximum yielding plants in the greenhouse experiment had higher leaf P, Fe and Zn concentrations. The maximum yielding plants may have had more efficient nutrient absorption systems. More extensive root development often accompanies increased top growth and has been associated with increased tissue concentrations of some elements (43, 44, 53, 79, 80, 89), however, N applications with nitrapyrin reduced plant size and increased leaf P, Fe and Zn concentrations in the greenhouse. Higher P, Fe and Zn concentrations from NH_4 -nitrapyrin applications in this case may partially be due to a "dilution effect" because of smaller plant size, but this alone would not account for all of the concentration increase.

It was determined that NH_4 -nitrapyrin applications did produce plants with higher leaf concentrations of P, Fe and Zn when concentrations from equal sized plants were compared. A trend for leaf Fe and Zn concentration increases from NH_4 -nitrapyrin applications was observed in the greenhouse but was statistically not significant. The significance of leaf P, Fe and Zn increases due to N applications with nitrapyrin was lowered by the inhibitory effect of nitrapyrin on leaf concentrations of these elements in the absence of an NH_4 application.

In the 1975 field experiment, spinach tissue was sampled at 4 growth stages. The concentration of P in leaves was increased the most by NH_4 -nitrapyrin applications 28 days from planting. This may be an important effect because of the importance of P to early plant growth and development. Increase in leaf concentrations of Fe and Zn with NH_4 -nitrapyrin applications were similar at all sample times. Generally, leaf P, Fe and Zn concentrations, as well as soil NH_4 -N concentrations, increased with increasing rates of nitrapyrin. Although not reported in this study, the level of soil NH_4 did not correlate well with leaf P, Fe and Zn content because of extreme NH_4 -N and leaf P, Fe and Zn concentration variability.

In the greenhouse and 1975 field experiment, smaller plants from the no N control treatments generally had the highest concentrations of P, Fe and Zn. The only exception to this was leaf Fe

concentrations in the 1975 field experiment which were highest from the low N applications. Higher leaf concentrations of these elements from no N control treatments is attributed to a "dilution effect."

Leaf P and Fe concentrations from the 1975 field experiment were at a measured maximum 28 days from planting while Zn concentrations were at a measured maximum 42 days from planting. These differences between P and Fe, and Zn concentrations cannot be explained.

Leaf P, Fe and Zn concentrations were increased by nitrapyrin in the absence of NH_4 applications in the 1975 field experiment. This stimulatory effect was directly opposite the effect of nitrapyrin without NH_4 applications in the greenhouse experiment in which concentrations of these elements decreased. It was expected that nitrapyrin would only influence the leaf content of P, Fe and Zn in the presence of NH_4 . Neither the inhibitory effect of nitrapyrin alone observed in the greenhouse experiment nor the stimulatory effect of nitrapyrin alone in the 1975 field experiment was expected and cannot be explained.

The effect of NH_4 -nitrapyrin treatments on leaf concentrations of P, Fe and Zn was related to the relative amounts of these elements required by plants. Phosphorous is absorbed by plants in much greater quantities than Fe or Zn and the effects of NH_4 -nitrapyrin applications on leaf P concentrations were much greater. Iron is required by plants in greater quantities than Zn and the effect of NH_4 -nitrapyrin applications on leaf Fe concentrations was greater than with Zn.

All 3 experiments have indications that leaf concentrations of P, Fe and Zn were increased by NH_4 -nitrapyrin applications. Yield increases from the 1974 and 1975 field experiments with NH_4 -nitrapyrin treatments have been attributed to either the soil N form or combinations of N forms, or increased soil N retention, but increased P, Fe and Zn absorption from these treatments cannot entirely be ruled out as possible reasons for increased yields in these alkaline soils. Analysis of these field soils indicated that these elements were not limiting, therefore increased yields were most likely not due to increased absorption of P, Fe or Zn. The fact that leaf concentrations of P, Fe and Zn can be increased by suppressing nitrification with NH_4 applications indicates possibilities of using nitrapyrin to correct soil P, Fe or Zn deficiencies in alkaline soils.

SUMMARY

The effect of the nitrification suppressant, nitrapyrin, in conjunction with $(\text{NH}_4)_2\text{SO}_4$, on spinach growth in alkaline soil was determined from 2 field experiments and one greenhouse experiment. Parameters measured were soil NH_4 -N and NO_3 -N concentrations, spinach yield, leaf color and leaf tissue concentrations of P, Fe and Zn.

Nitrapyrin rates as low as 140 g/ha effectively suppressed nitrification in the field. Increasing nitrapyrin application rates suppressed nitrification for longer periods of time. Apparently no leaching occurred in the 1974 field experiment resulting in higher than expected soil NO_3 -N concentrations. Leaching was apparently experienced in the 1975 field experiment. Increasing nitrapyrin application rates produced higher levels of soil NH_4 -N throughout this experiment. Highly concentrated soil NH_4 -N conditions resulted when 1.5 ppm nitrapyrin suppressed nitrification for the length of the experiment in the greenhouse.

Ammonium-nitrapyrin applications increased spinach yields in both 1974 and 1975 field experiments. Maximum yields from NH_4 -nitrapyrin applications in 1974 were attributed to a favorable soil NO_3 -N and NH_4 -N balance since NO_3 leaching was reduced. The nitrapyrin application rate resulting in more concentrated soil NH_4

produced better plant growth than NO_3 alone in this experiment. Increased yields in the 1975 field experiment were attributed to increased soil N retention. Ammonium-nitrapyrin applications severely reduced plant growth and induced NH_4 toxicity symptoms in the greenhouse. The greenhouse medium did not support plant growth but concentrated NH_4 as did the 1974 field experiment.

Spinach leaf color was darker green from NH_4 -nitrapyrin treatments in all experiments, however, green leaf color was only increased when nitrapyrin was applied with $(\text{NH}_4)_2\text{SO}_4$.

Nitrapyrin treated $(\text{NH}_4)_2\text{SO}_4$ increased leaf concentrations of P and Fe in all 3 experiments. Ammonium-nitrapyrin applications did tend to increase Zn concentrations in the greenhouse experiment and 1975 field experiment but to a much lesser degree than P and Fe. The concentration of these elements were generally increased only when nitrapyrin was applied with $(\text{NH}_4)_2\text{SO}_4$, however, leaf P, Fe and Zn concentrations were unexpectedly decreased in the greenhouse experiment and increased in the 1975 field experiment by nitrapyrin in the absence of $(\text{NH}_4)_2\text{SO}_4$ for reasons unknown.

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APPENDIX

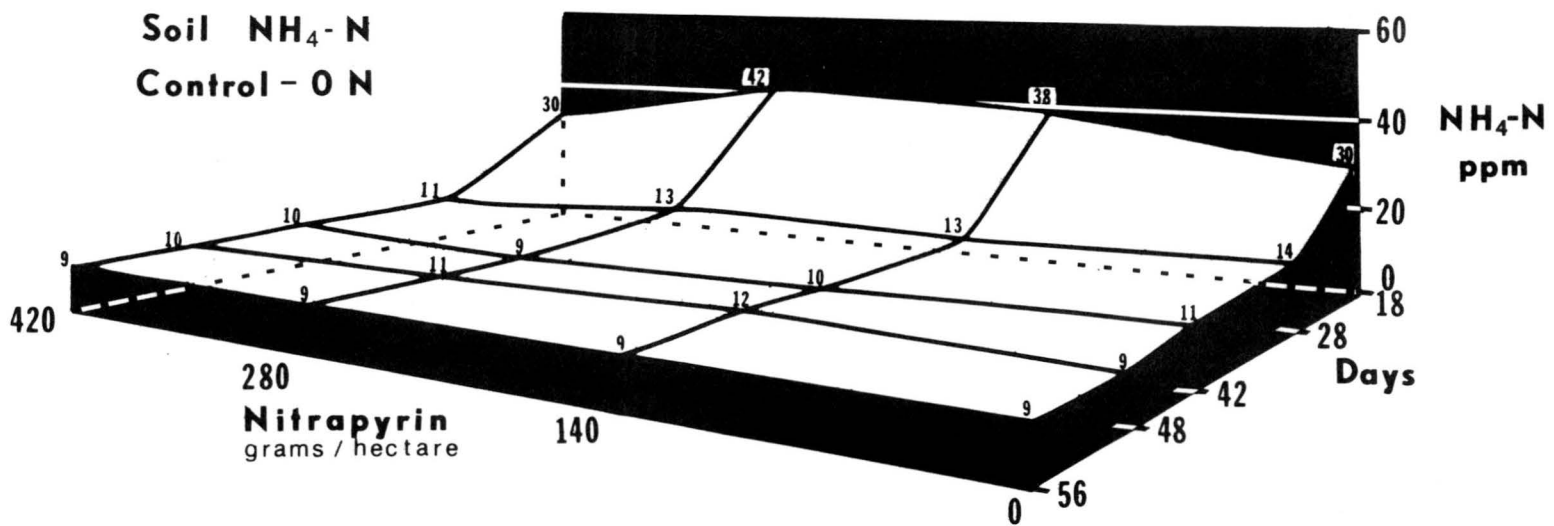


Figure 1. Mean soil $\text{NH}_4\text{-N}$ concentrations (ppm) for nitrapyrin application rates at 0 N 18, 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean soil $\text{NH}_4\text{-N}$ concentrations.

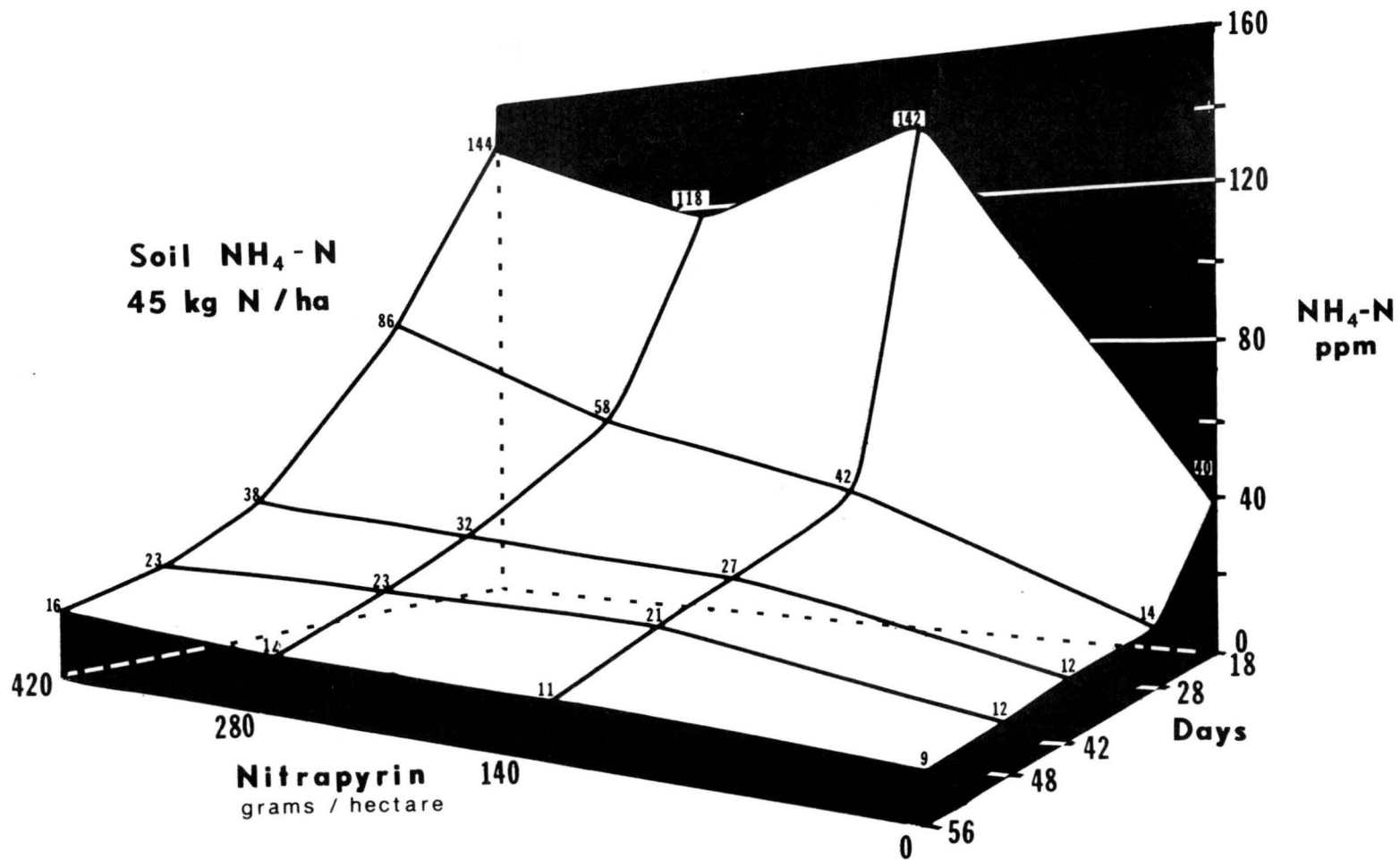


Figure 2. Mean soil NH₄-N concentrations (ppm) for nitrapyrin application rates at 45 kg N/ha 18, 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean soil NH₄-N concentrations.

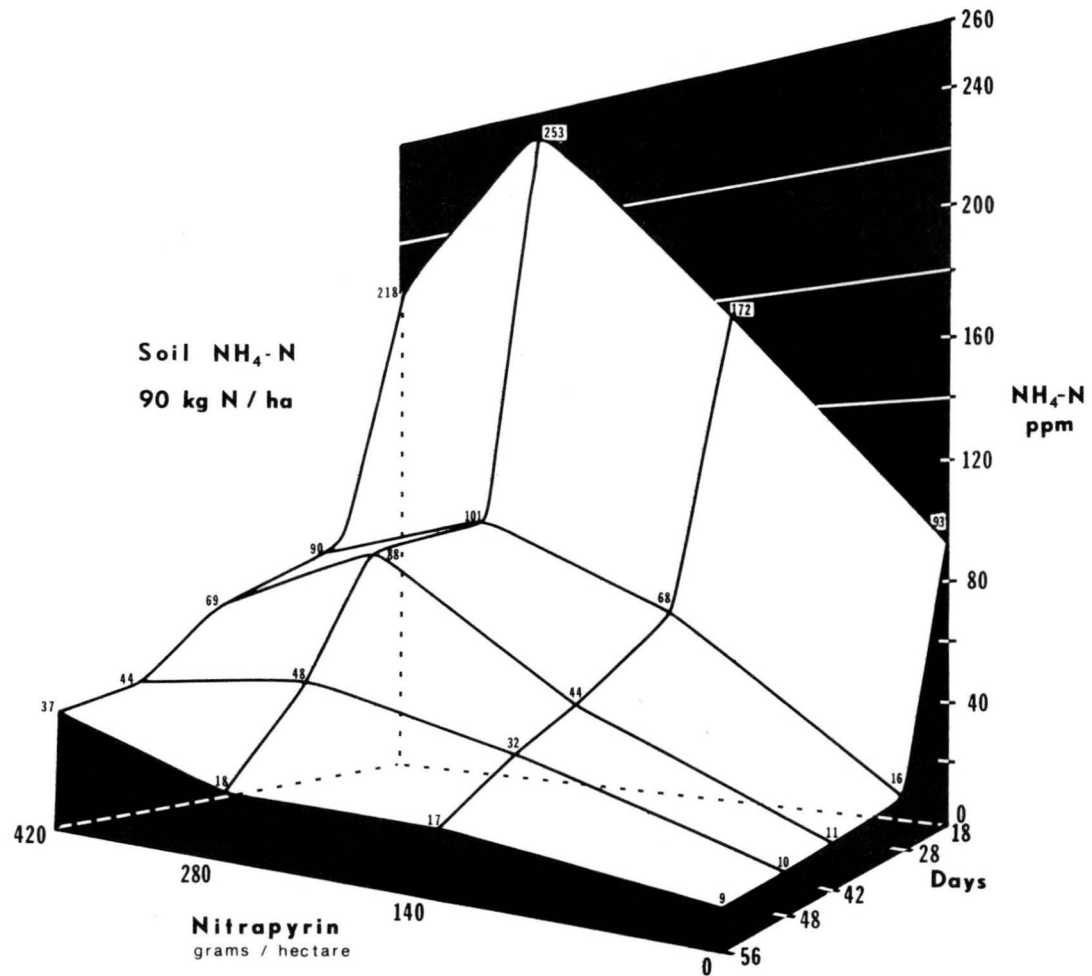


Figure 3. Mean soil NH₄-N concentrations (ppm) for nitrapyrin application rates at 90 kg N/ha 18, 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean soil NH₄-N concentrations.

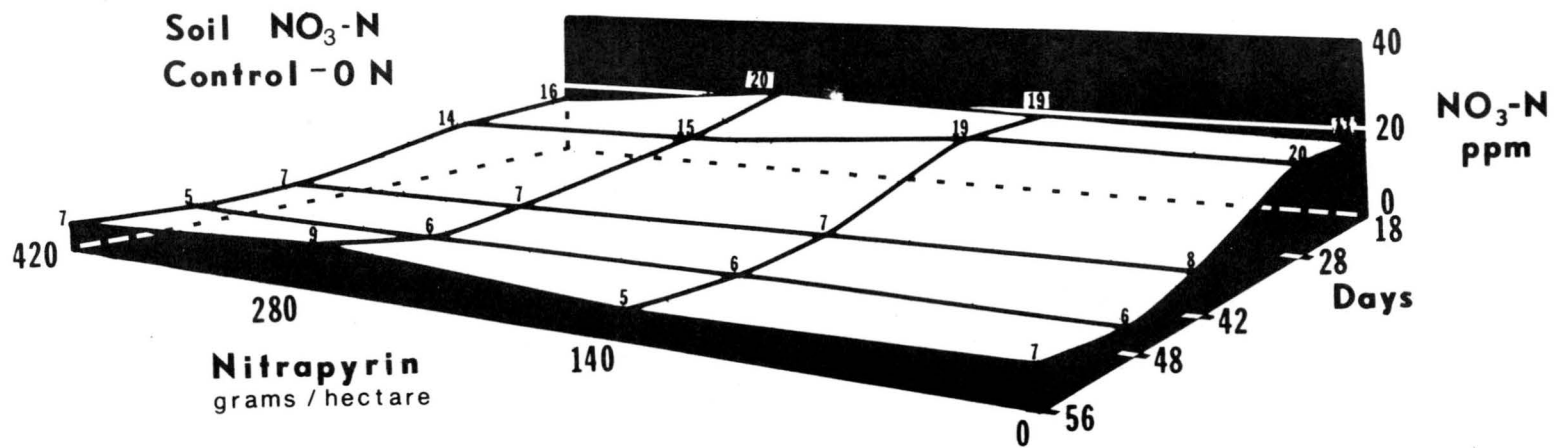


Figure 4. Mean soil NO₃-N concentrations (ppm) for nitrapyrin application rates at 0 N 18, 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean soil NO₃-N concentrations.

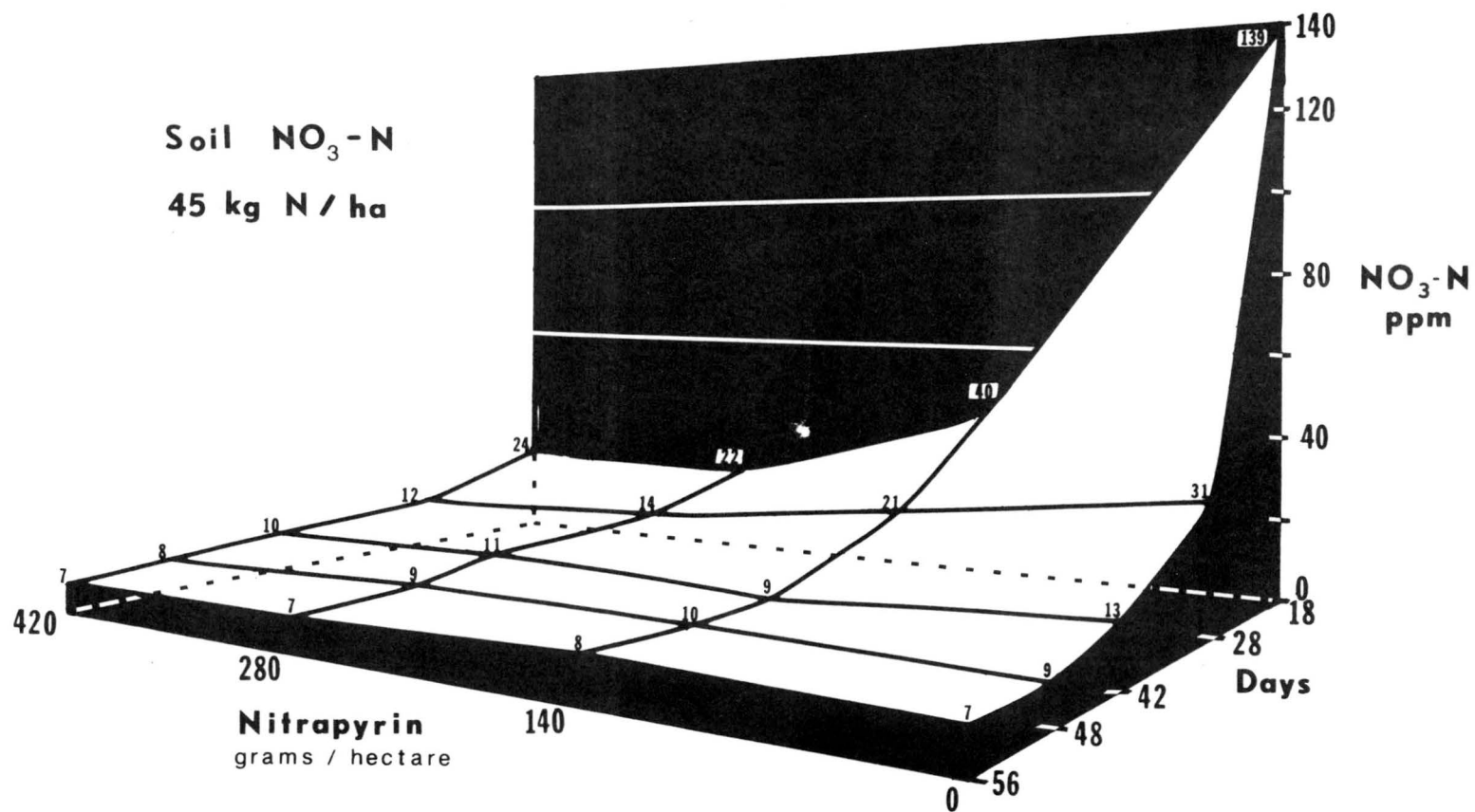


Figure 5. Mean soil NO₃-N concentrations (ppm) for nitrapyrin application rates at 45 kg N/ha 18, 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean soil NO₃-N concentrations.

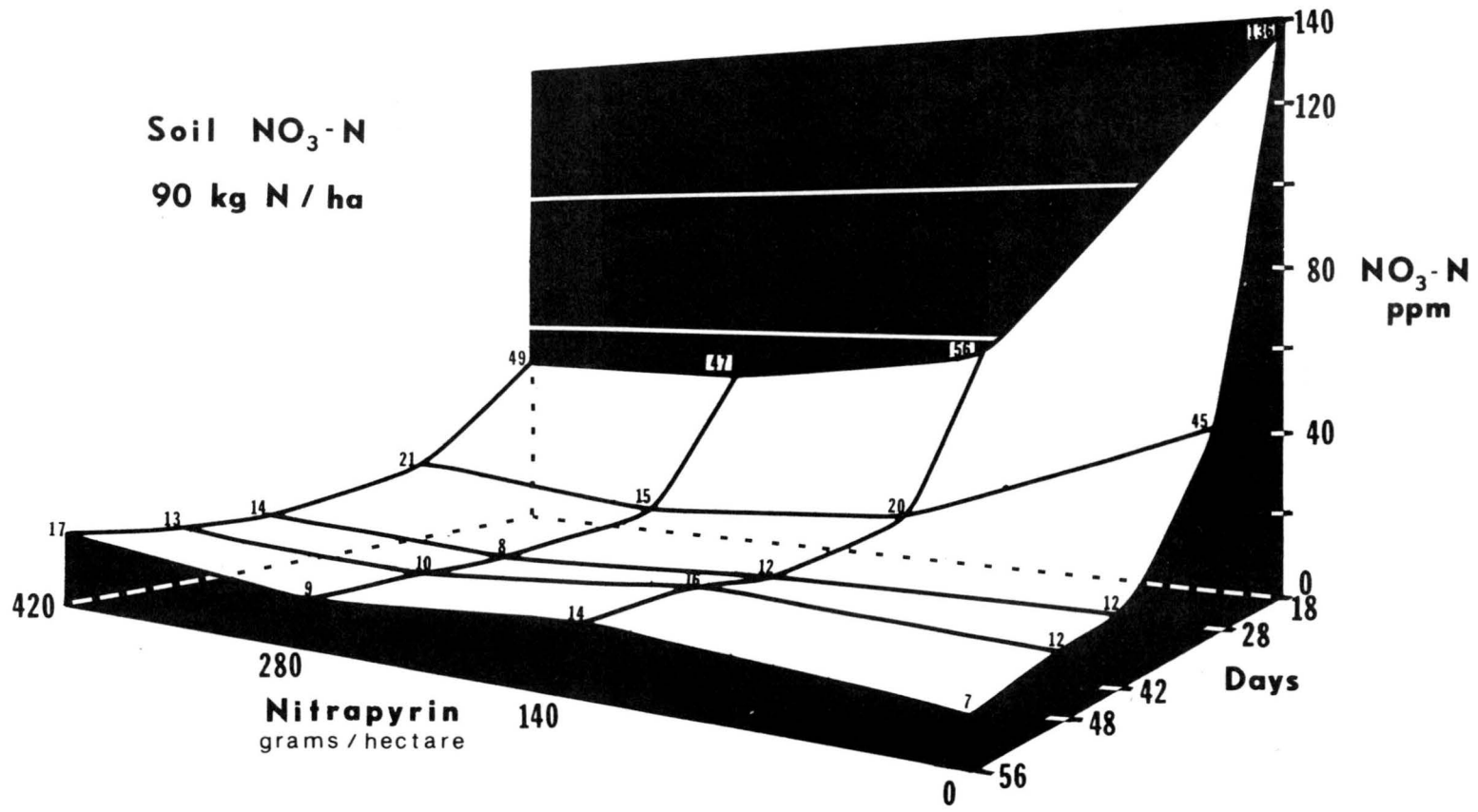


Figure 6. Mean soil NO₃-N concentrations (ppm) for nitrapyrin application rates at 90 kg N/ha 18, 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean soil NO₃-N concentrations.

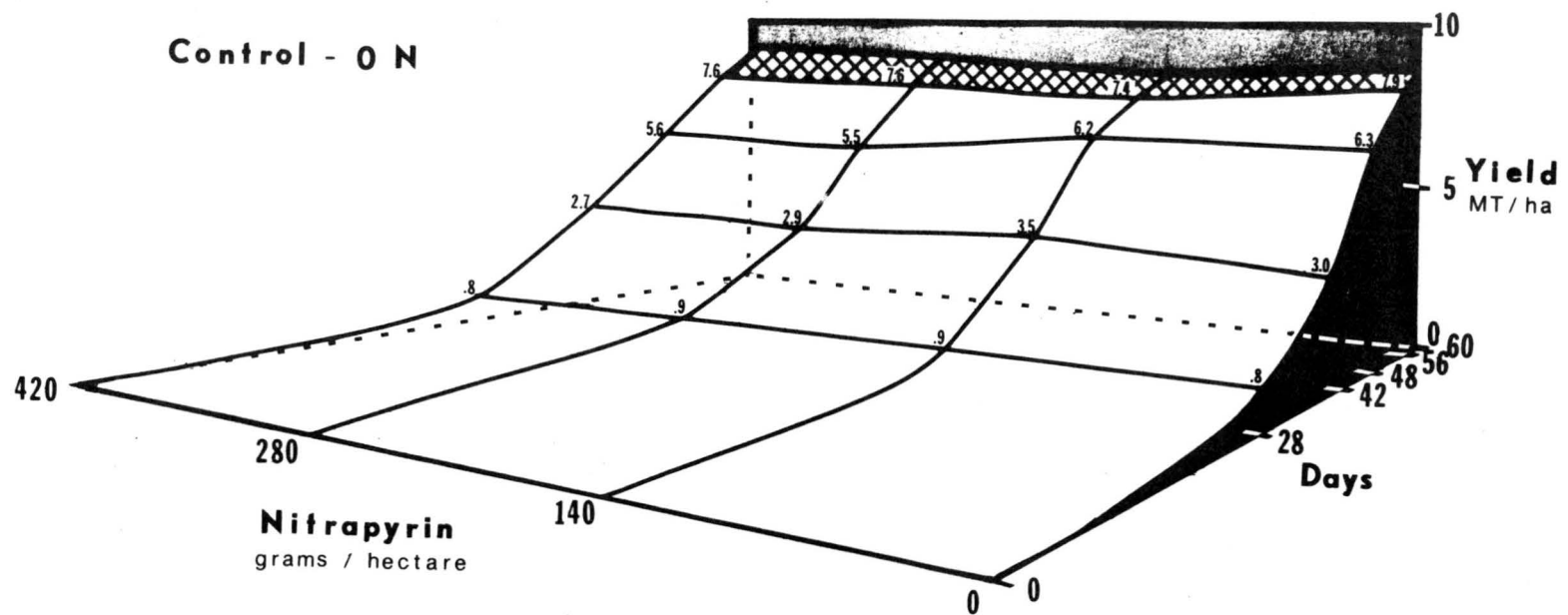


Figure 7. Mean spinach yields (MT/ha) for nitrapyrin application rates at 0 N 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean spinach yields. Cross-hatch indicates forecasted spinach yields 60 days from planting.

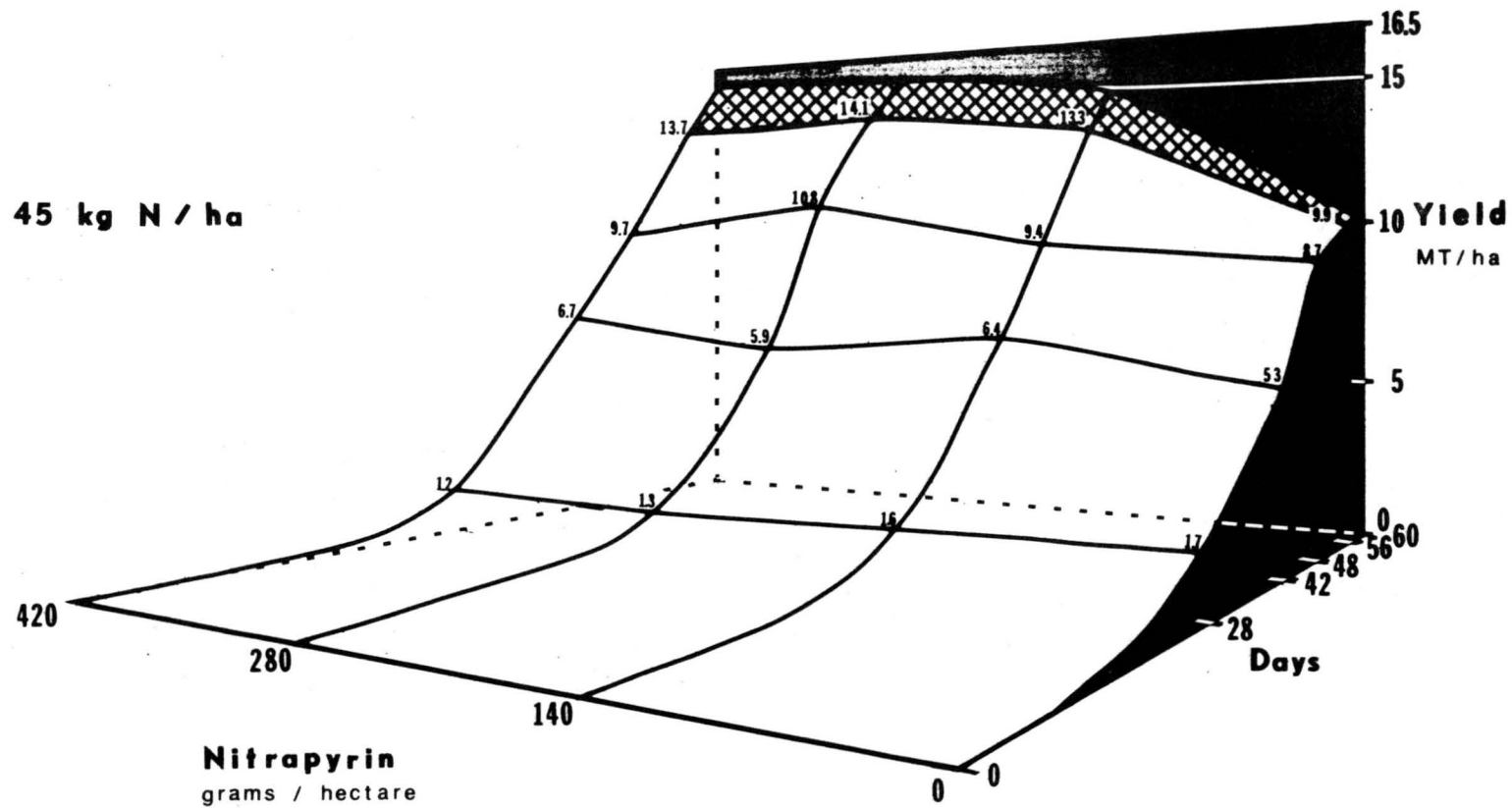


Figure 8. Mean spinach yields (MT/ha) for nitrapyrin application rates at 45 kg N/ha 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean spinach yields. Cross-hatch indicates forecasted spinach yields 60 days from planting.

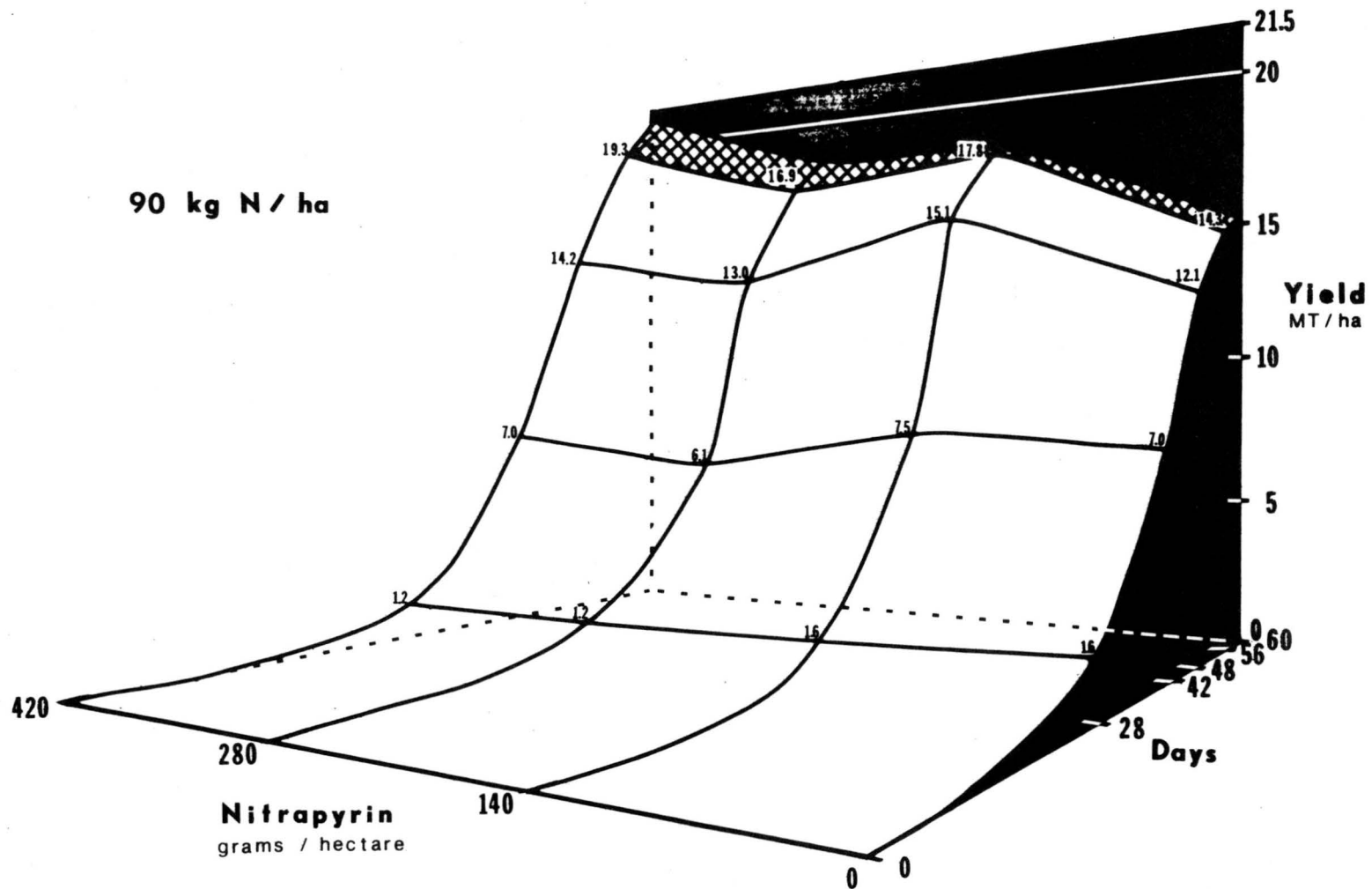


Figure 9. Mean spinach yields (MT/ha) for nitrapyrin application rates at 90 kg N/ha 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean spinach yields. Cross-hatch indicates forecasted spinach yields 60 days from planting.

Figure 10. The row to the left of center is 90 kg N/ha without nitrapyrin while the row to the right of center is the same N application rate with 140 g nitrapyrin/ha.

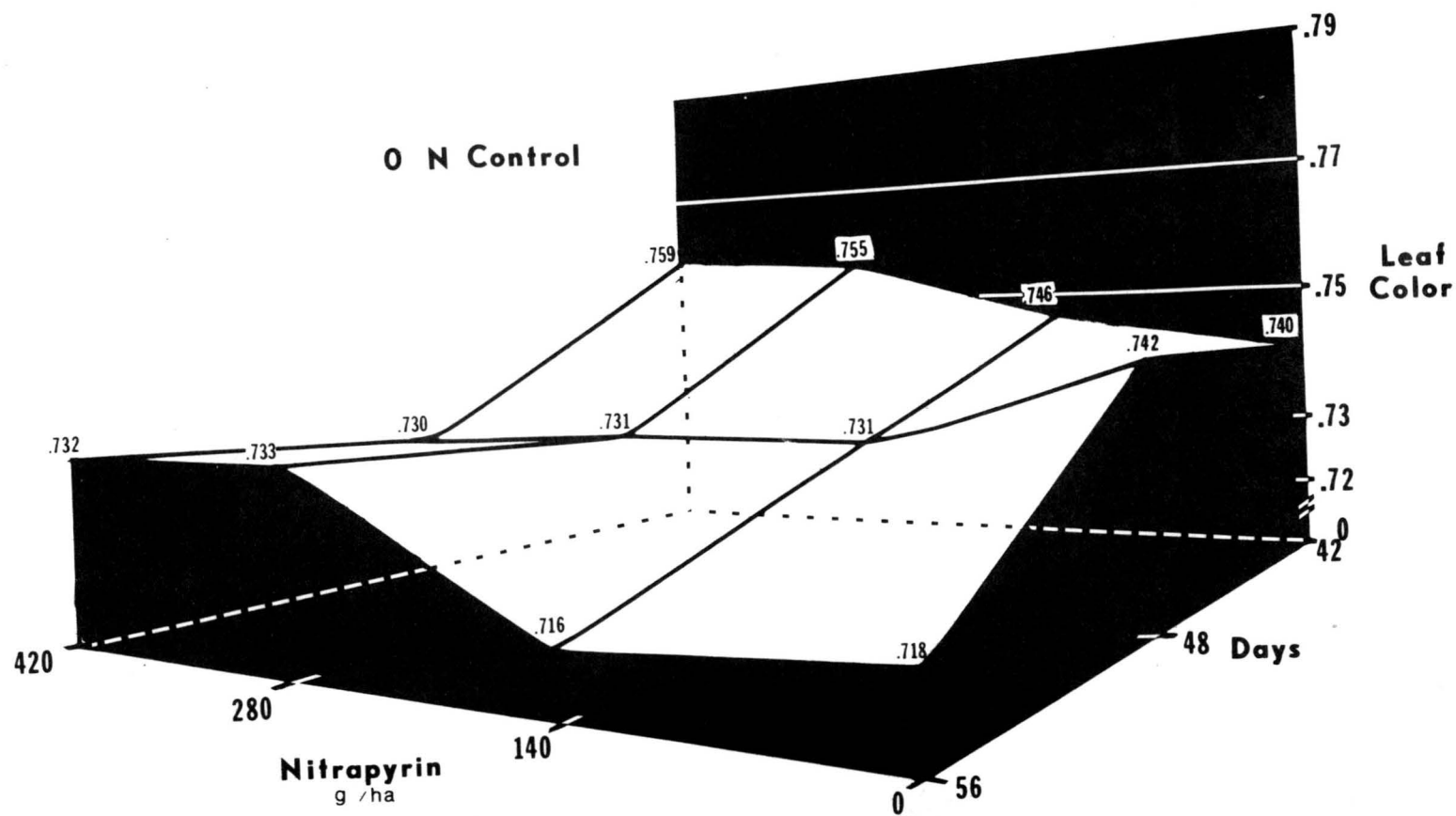


Figure 11. Mean leaf light absorption values (leaf color) for nitrapyrin application rates at 0 N 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean light absorption values.

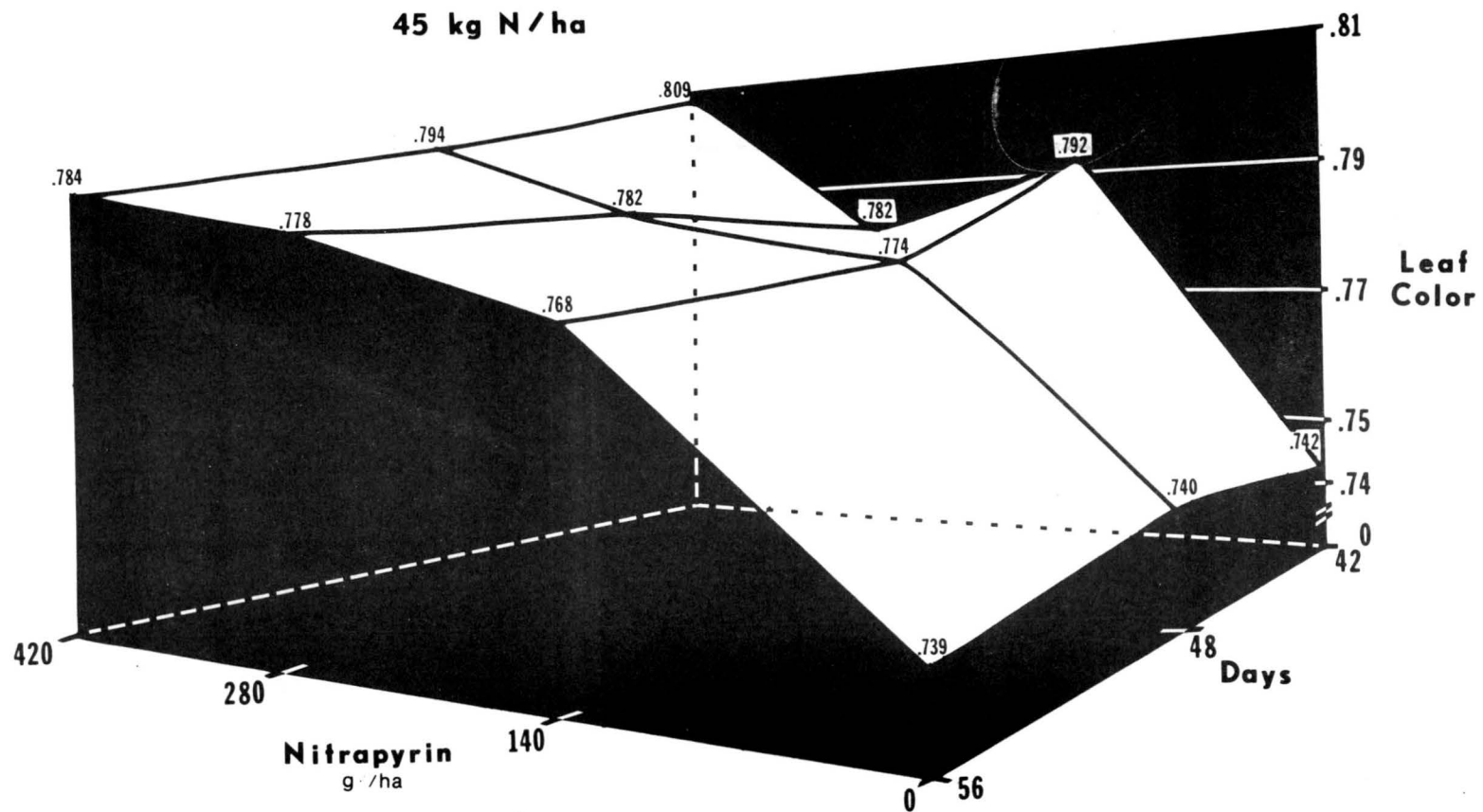


Figure 12. Mean leaf light absorption values (leaf color) for nitrapyrin application rates at 45 kg N/ha 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean light absorption values.

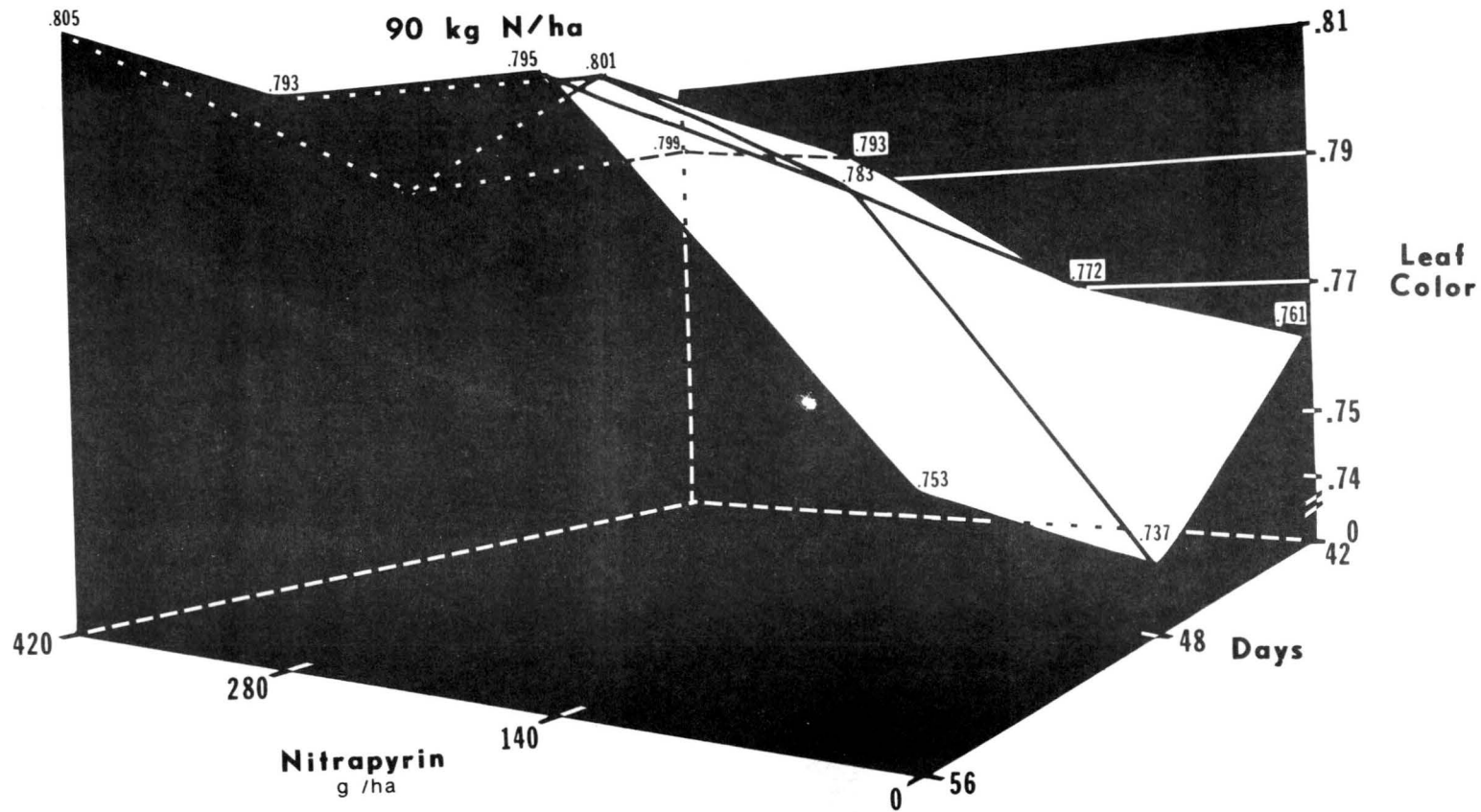


Figure 13. Mean leaf light absorption values (leaf color) for nitrapyrin application rates at 90 kg N/ha 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean light absorption values.

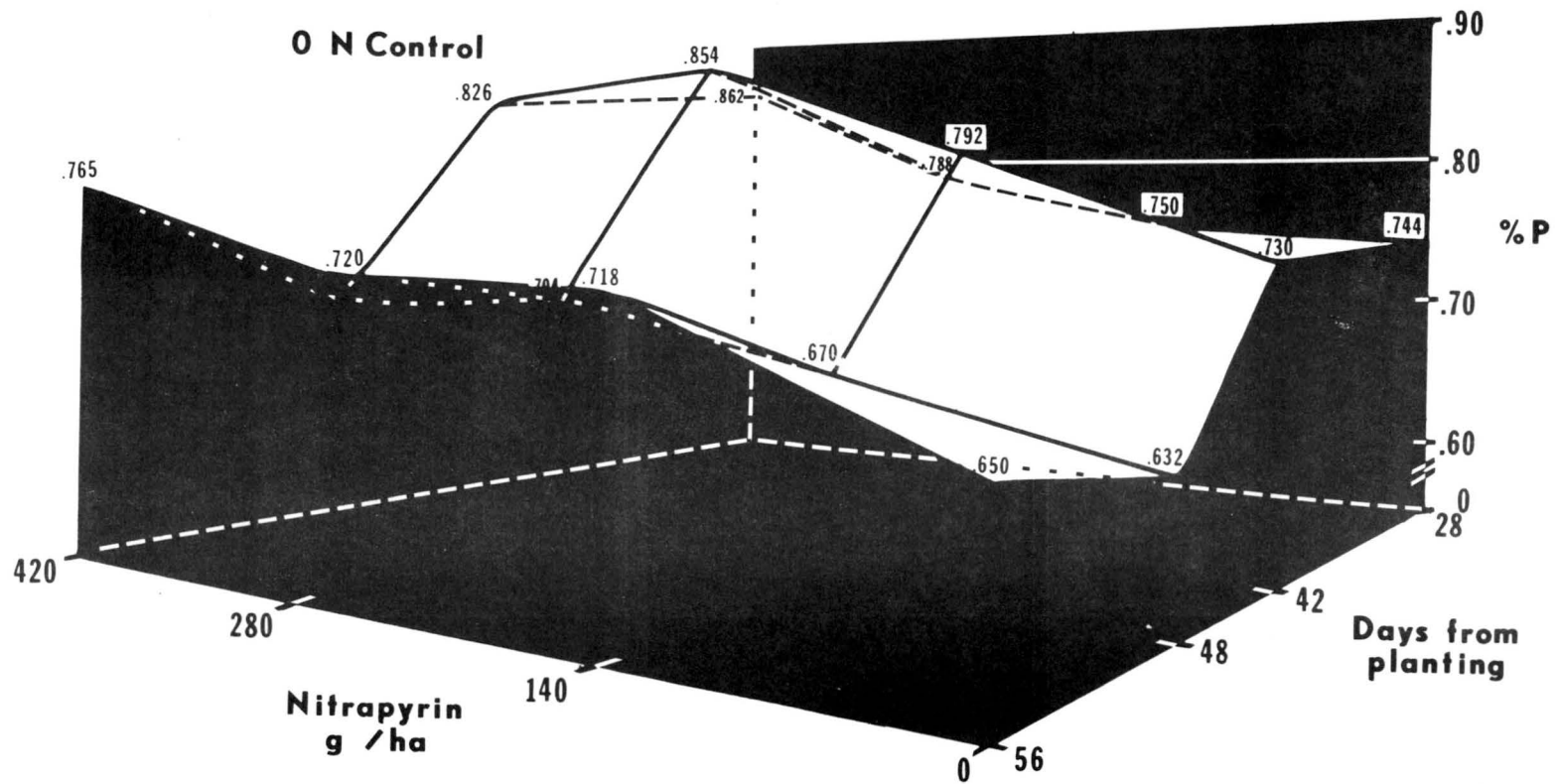


Figure 14. Mean leaf P concentrations (%) for nitrapyrin application rates at 0 N 28, 42, 48, and 56 days from planting. Numbers at the intersection of reference lines are mean P concentrations.

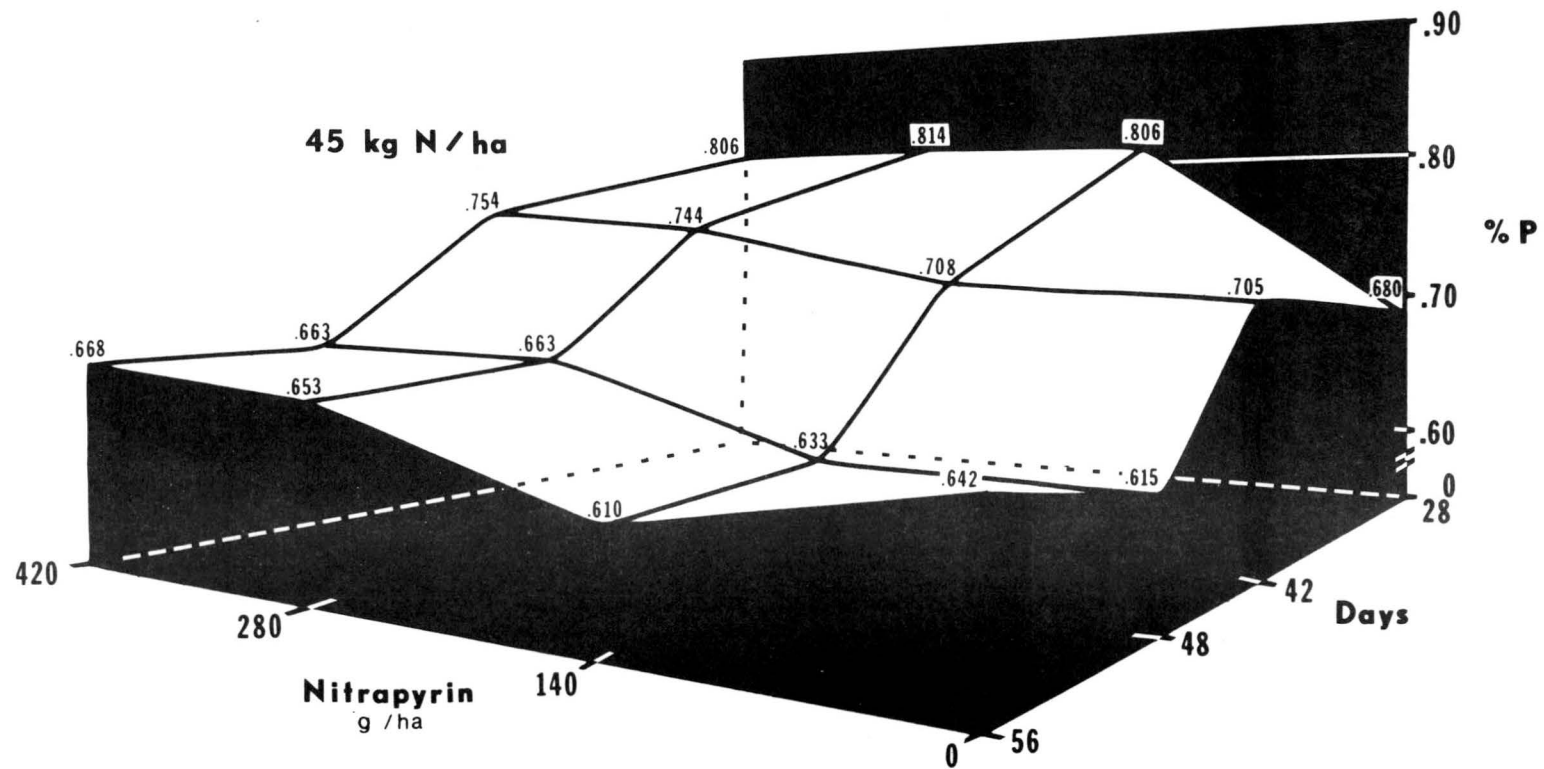


Figure 15. Mean leaf P concentrations (%) for nitrapyrin application rates at 45 kg N/ha 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean P concentrations.

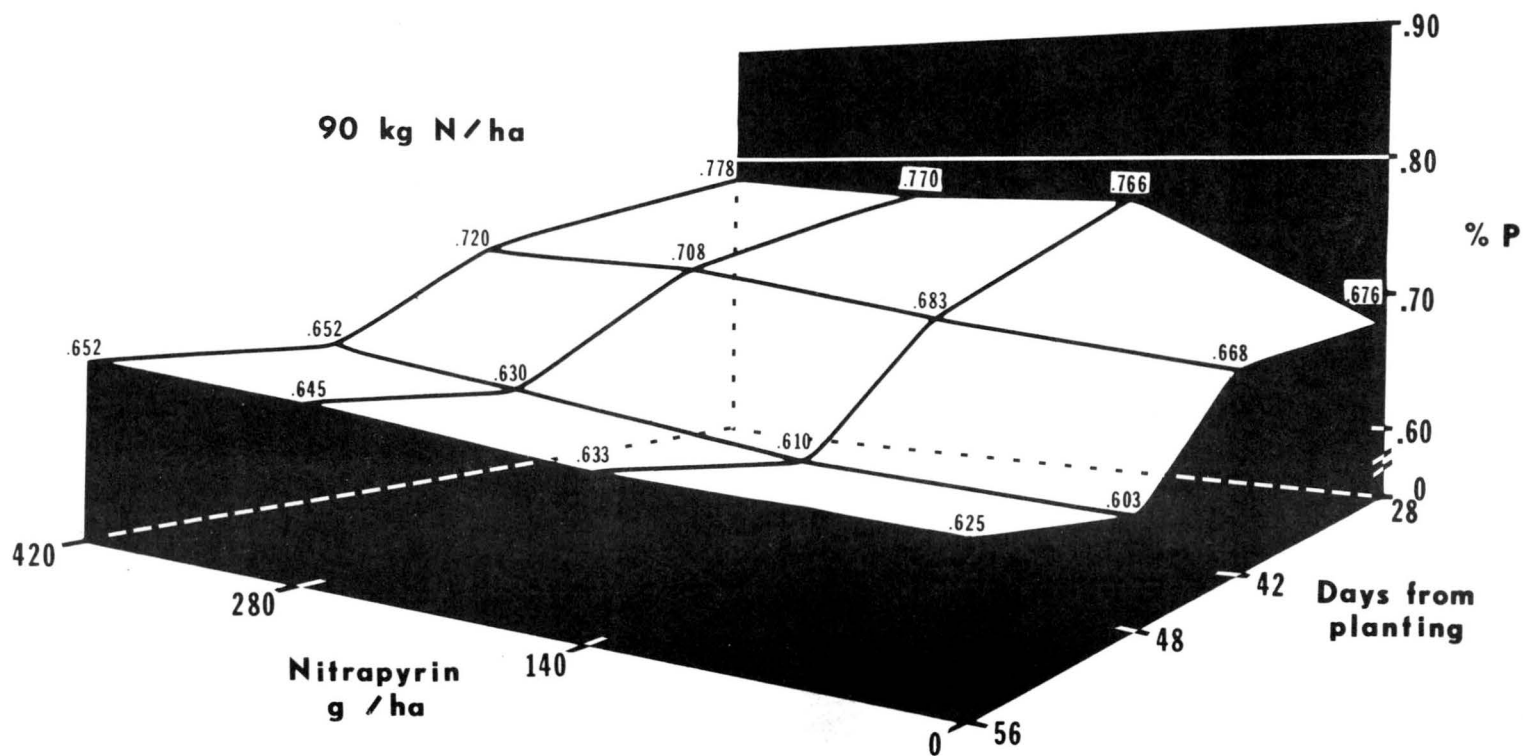


Figure 16. Mean leaf P concentrations (%) for nitrapyrin application rates at 90 kg N/ha 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean P concentrations.

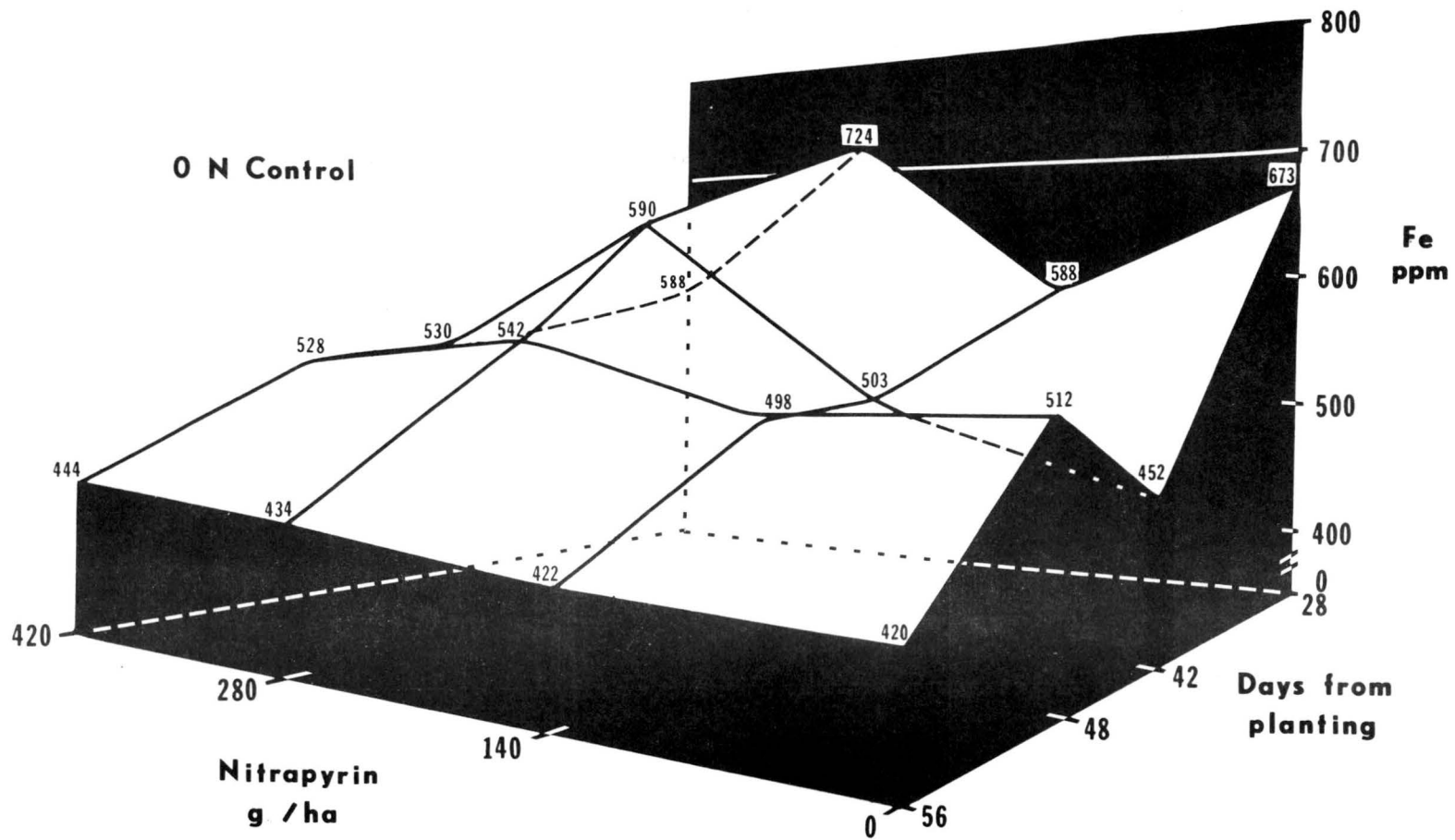


Figure 17. Mean leaf Fe concentrations (ppm) for nitrapyrin application rates at 0 N 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean Fe concentrations.

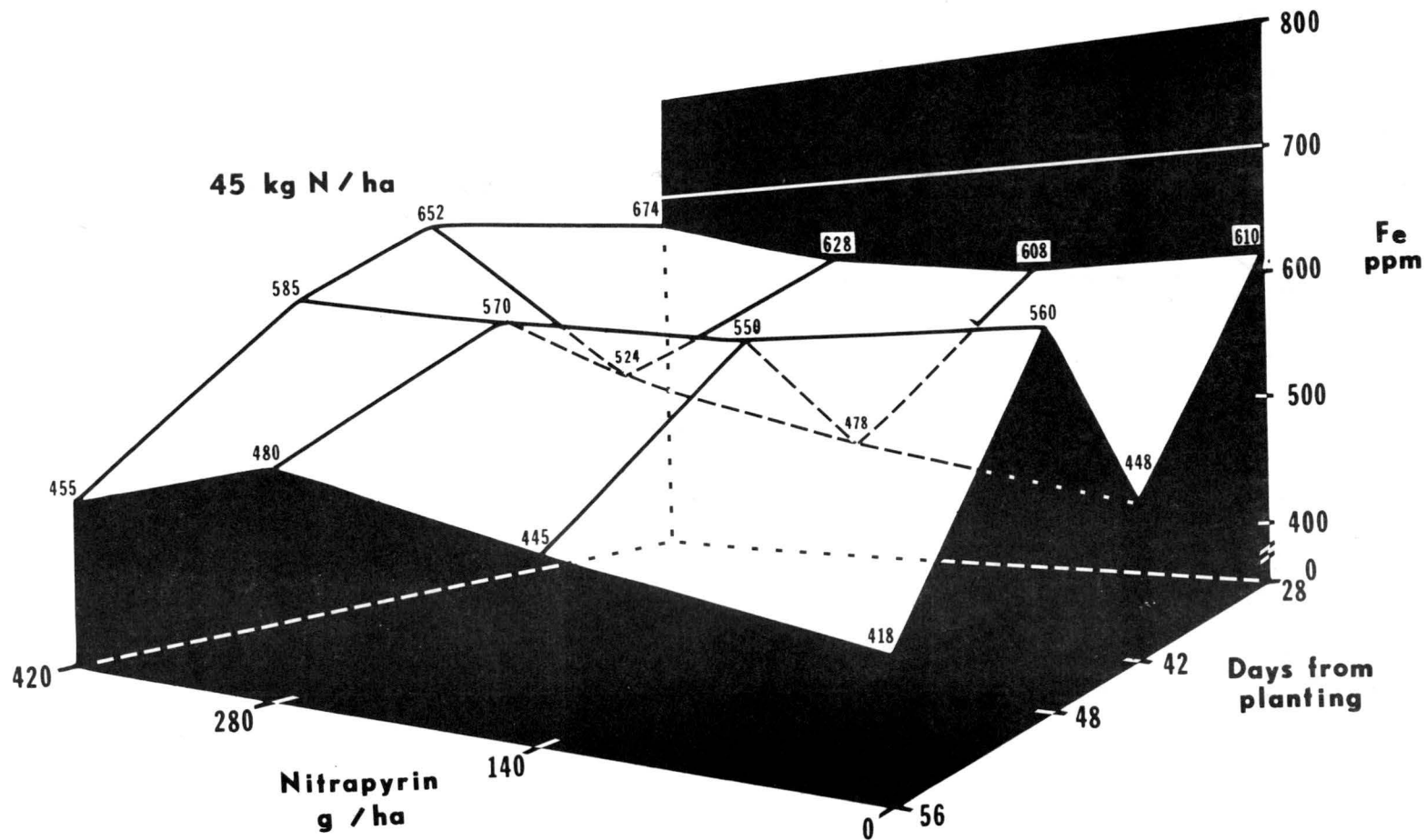


Figure 18. Mean leaf Fe concentrations (ppm) for nitrapyrin application rates at 45 kg N/ha 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean Fe concentrations.

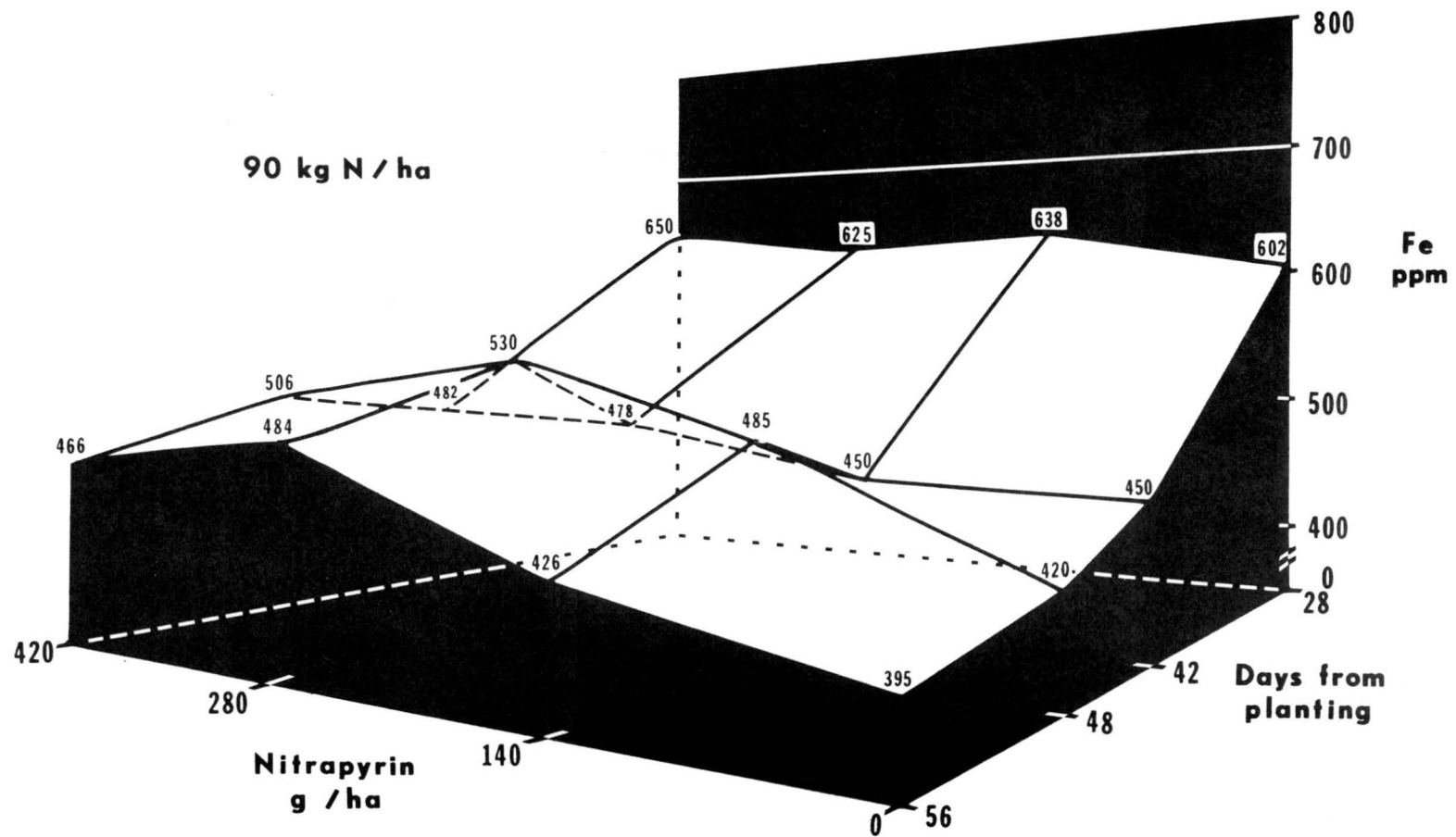


Figure 19. Mean leaf Fe concentrations (ppm) for nitrapyrin application rates at 90 kg N/ha 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean Fe concentrations.

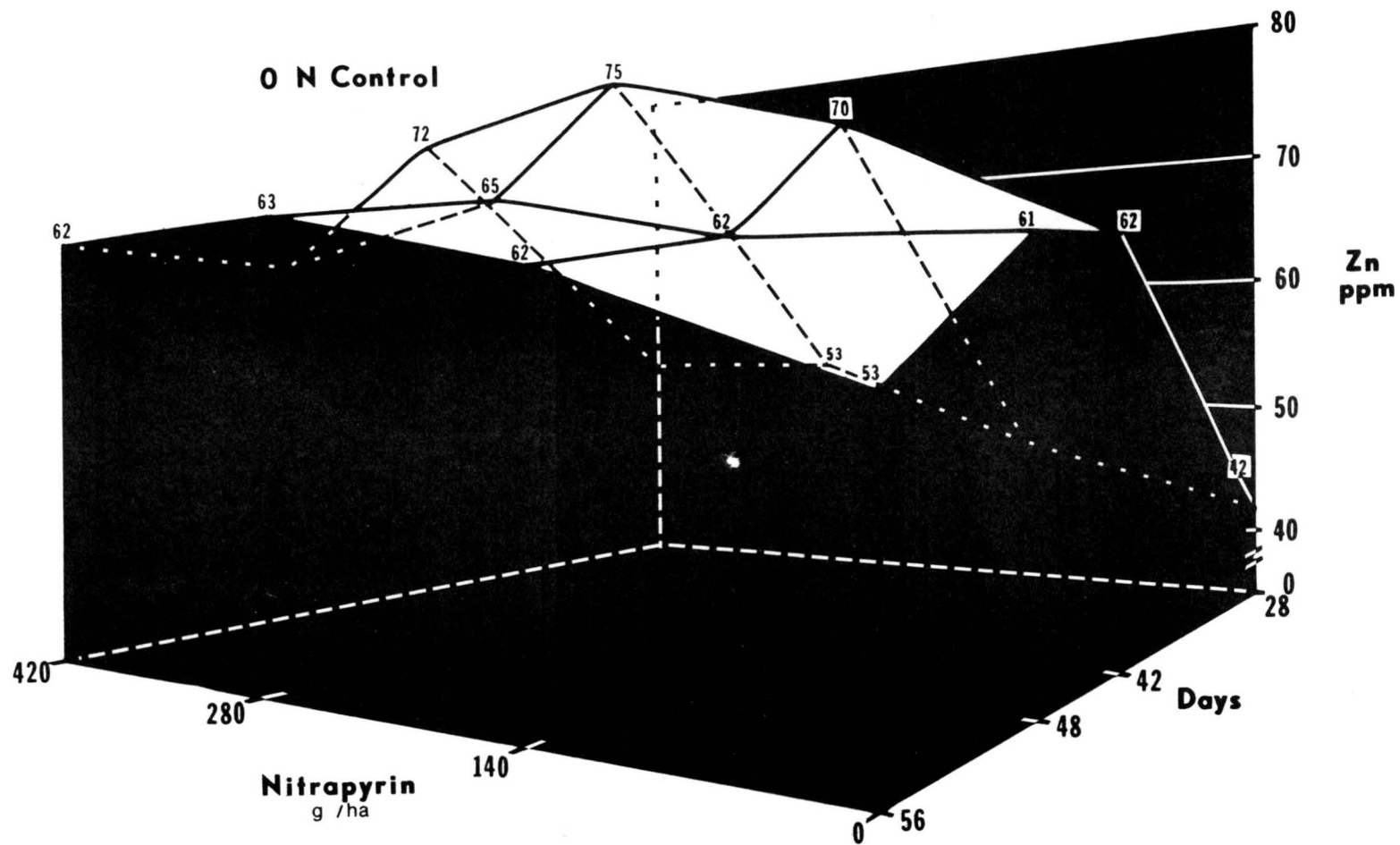


Figure 20. Mean leaf Zn concentrations (ppm) for nitrapyrin application rates at 0 N 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean Zn concentrations.

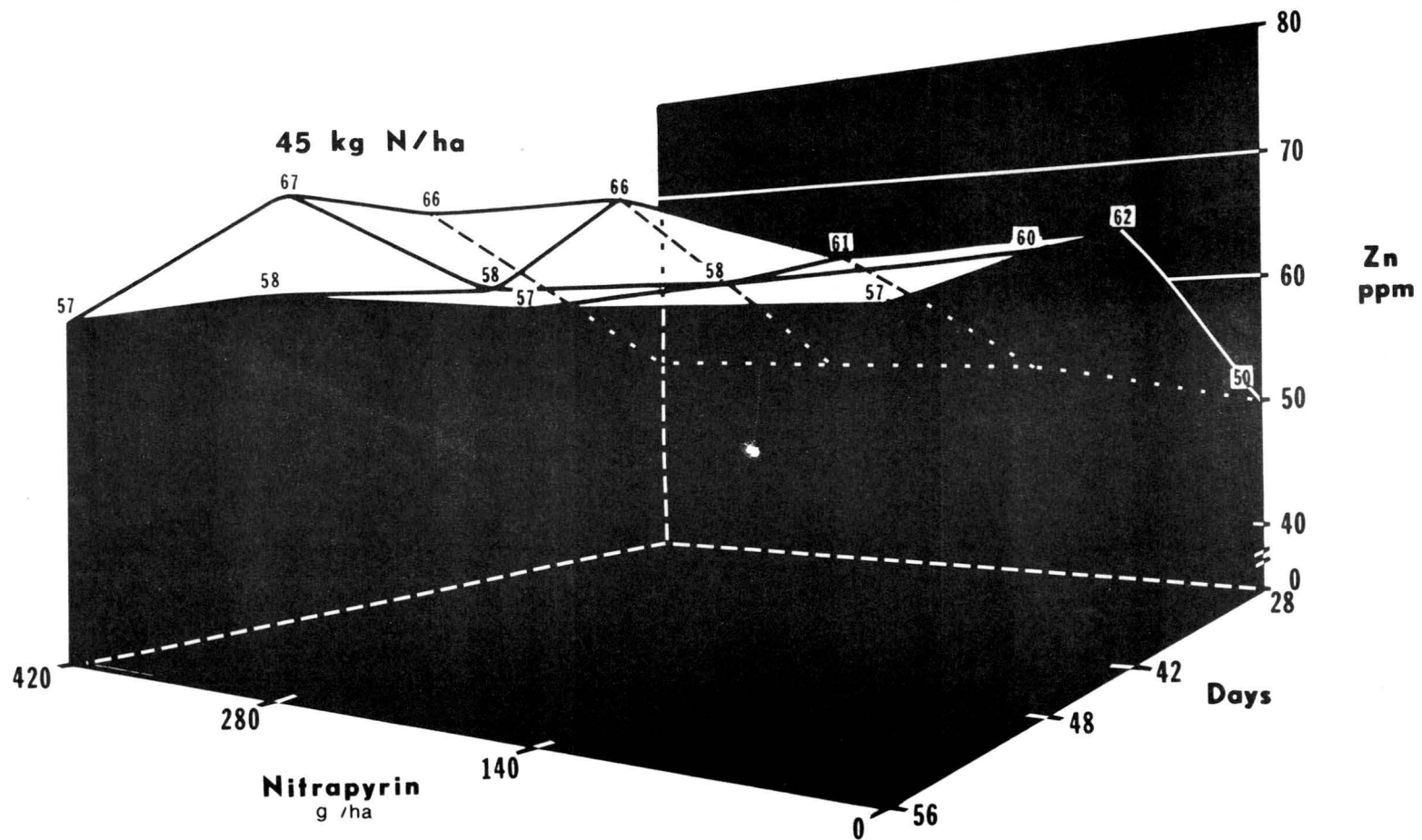


Figure 21. Mean leaf Zn concentrations (ppm) for nitrapyrin application rates at 45 kg N/ha 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean Zn concentrations.

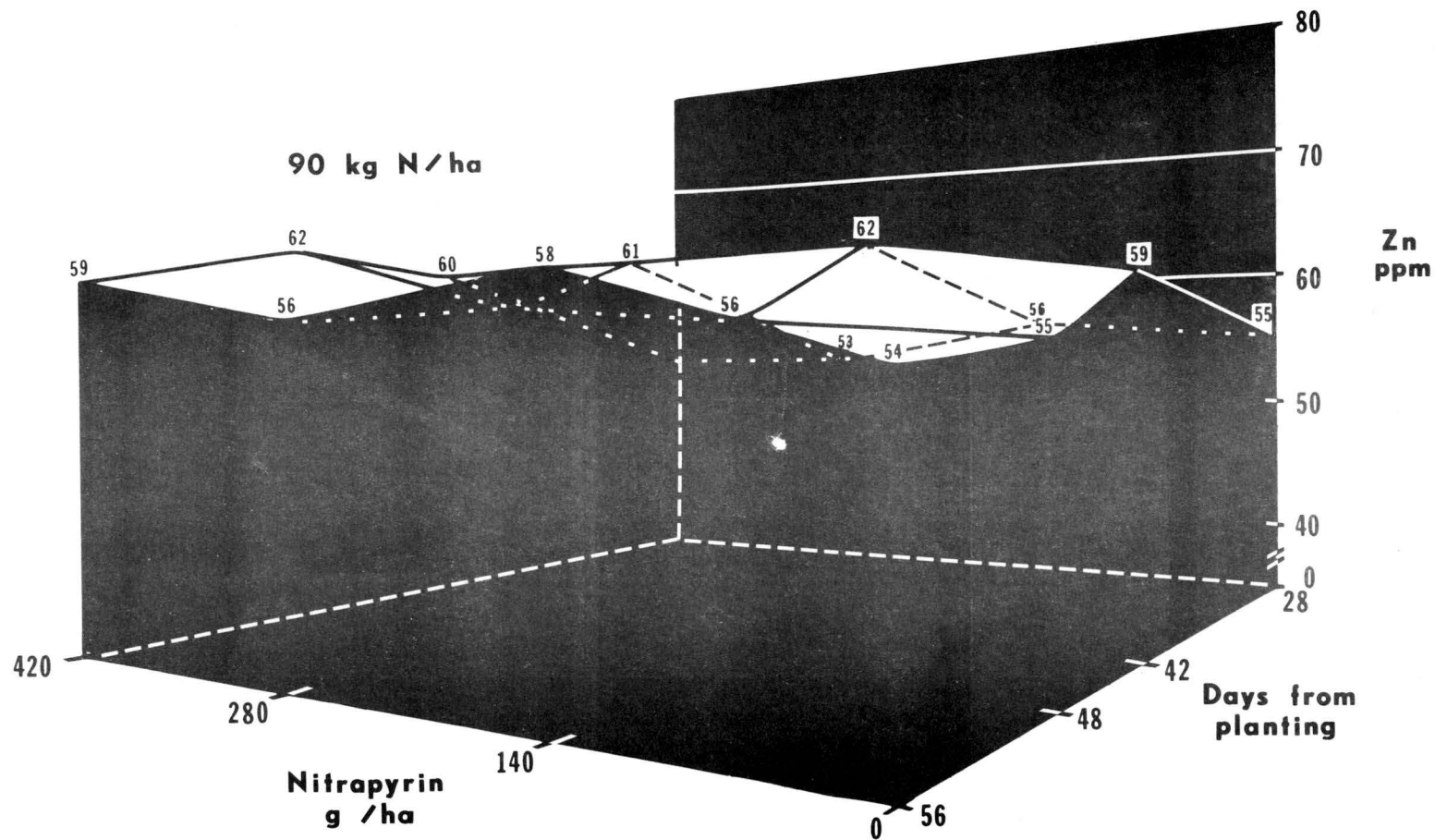


Figure 22. Mean leaf Zn concentrations (ppm) for nitrapyrin application rates at 90 kg N/ha 28, 42, 48 and 56 days from planting. Numbers at the intersection of reference lines are mean Zn concentrations.