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**N15-TRACER STUDIES OF THE TRANSFORMATIONS, MOVEMENT, AND  
RECOVERY OF ANHYDROUS AMMONIA-DERIVED N IN THE ROOTING  
ZONE OF CORN**

*Iowa State University*

**Ph.D. 1986**

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<sup>15</sup>N-tracer studies of the transformations, movement, and recovery  
of anhydrous ammonia-derived N in the rooting zone of corn

by

Charles Anthony Sanchez

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of the  
Requirements for the Degree of  
DOCTOR OF PHILOSOPHY

Department: Agronomy  
Major: Soil Fertility

Approved:

Signature was redacted for privacy.

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For the Graduate College

Iowa State University  
Ames, Iowa

1986

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## INTRODUCTION

Agronomists always have known that a substantial portion of the fertilizer N applied to soils is lost by processes other than plant uptake. Although they always have tried to identify practices that reduce these losses, there are several reasons why these efforts must be intensified in Iowa. First, Iowa farmers are currently using more than 10 times the amount of N fertilizer they were using about three decades ago. They now are applying about one million tons of fertilizer N to Iowa soils each year. Second, the current economic situation requires farmers to invest in no more fertilizer than is needed. Third, there is mounting concern that some of the N that escapes from agricultural soils may contaminate groundwater supplies. There is a growing awareness that our groundwater is a valuable resource that must be protected.

It is well established that N can be lost from soils by several different processes. Denitrification is a biological process in which nitrate or nitrite is reduced to gaseous forms of N that escape to the atmosphere. Ammonia volatilization is a chemical process by which fertilizer N can escape to the atmosphere. Leaching is a physical process by which fertilizer N can move below the rooting zone with water that infiltrates through the soil. Although there have been many studies to elucidate the mechanisms by which each of these processes occur and it generally is accepted that each of these processes can result in substantial losses of fertilizer N under appropriate conditions, there have been few studies designed to evaluate the

relative importance of each of these processes during corn production in Iowa.

There are several reasons why there have been few studies to assess the relative importance of the various processes by which N is lost from soils under field conditions. One of the most important is that it is practically impossible to measure the amounts of N lost by denitrification or leaching under most field conditions. Another is that, as soon as N fertilizers are applied to soils, they enter into transformations that result in a rapid mixing of fertilizer-derived N with soil-derived N. Because the amounts of soil-derived N greatly exceed the amounts of fertilizer N, these transformations often make it impossible to determine whether N is lost from soil or merely incorporated into the soil.

Largely because of these difficulties, the only reliable way to assess N losses from soils under field conditions is to apply  $^{15}\text{N}$ -labeled fertilizers and determine the amounts of fertilizer N that remain within the soil-plant system at selected times after fertilization. By determining recovery of fertilizer N in various fractions of the soil-plant system, it is possible to make inferences about the processes responsible for N losses and, therefore, about the management practices having the highest probability of minimizing these losses.

One of the most promising tools for reducing losses of N fertilizers are nitrification inhibitors (Keeney, 1980; Meisinger et al., 1980; Hauck, 1983). These compounds inhibit the rapid oxidation of

ammonium to nitrate by the nitrifying organisms in soil (Goring, 1962a, b) and thereby reduce the potential for losses of fertilizer N by leaching and denitrification. Although nitrapyrin (a commercially available nitrification inhibitor) clearly inhibits nitrification in soils, there is little published data to show that use of this compound is cost-effective for crop producers in the western portion of the Corn Belt (Hergert and Wiese, 1980; Hoeft, 1984).

Despite the importance of N fertilizers for corn production in Iowa and the importance of knowing how much of this N is lost,  $^{15}\text{N}$  tracers have not been used to assess N losses during corn production in Iowa. The overall objective of the research conducted for this dissertation was to use  $^{15}\text{N}$  tracers to acquire a better understanding of the transformations and movement of anhydrous ammonia-derived N in soils and the response of corn to this N. Anhydrous ammonia was selected for study because it is the most widely used N fertilizer for corn production in Iowa. Nitrapyrin was included in this study because nitrification inhibitors are widely recognized as having potential for improving the efficiency of N fertilization and because nitrapyrin is the most widely used nitrification inhibitor.

The dissertation is divided into six parts. The first two parts are descriptions of methodology that was developed for these studies. The third part is an evaluation of the response of corn to anhydrous ammonia and nitrapyrin. The fourth part is an assessment of the amounts of fertilizer N recovered in soils and corn tissue over periods of one or more years. The fifth part examines the transformations and movement

of anhydrous ammonia-derived N in the rooting zone of corn during the growing season and the effects of nitrapyrin on these processes. The sixth part is an evaluation of the effects of nitrapyrin on denitrification of nitrate in soil-plant systems.

PART I. A METHOD FOR APPLICATION OF  $^{15}\text{N}$ -LABELED ANHYDROUS AMMONIA  
TO SMALL PLOTS

## INTRODUCTION

Although anhydrous ammonia is the most widely used N fertilizer in the Corn Belt, this material is often avoided by researchers because conventional ammonia applicators (tractor-drawn implements used in production agriculture) are difficult to calibrate for uniform rates of application on plots of the size normally used for research (Moraghan, 1980). These difficulties become especially acute when  $^{15}\text{N}$  tracers are used in studies of the transformations and movement of ammonia-derived N in soils. In such studies, the cost of the labeled fertilizers limits the size of field plots to a few (usually less than 5) square meters. The amount of ammonia applied to these small plots (often less than 0.1 L liquid ammonia) is less than the volume of the metering system and hoses on most conventional applicators.

Methods have been proposed (Papendick and Parr, 1965; Cochran et al., 1975; Bremner et al., 1981) that permit accurate application of small amounts of ammonia to soils. However, the methods of Papendick and Parr (1965) and Bremner et al. (1981) apply ammonia at point locations in soil, and it is essentially impossible to achieve a distribution of fertilizer that reproduces the distribution found when ammonia is applied in bands by conventional methods. The method of Cochran et al. (1975) is unsuitable for use in field studies. Furthermore, these methods cannot be used to apply mixtures of anhydrous ammonia and nitrification inhibitor (or other additives) because these methods involve a vaporization that separates ammonia from the

inhibitor.

A method that permits application of mixtures of  $^{15}\text{N}$ -labeled anhydrous ammonia and nitrification inhibitors is desirable because of the need (Keeney, 1980; Meisinger et al., 1980; Hauck, 1983) for evaluating nitrification inhibitors as tools for improving the efficiency of N fertilization. A method is described here that can be used to apply anhydrous ammonia with or without a nitrification inhibitor in bands to plots of the size often used in  $^{15}\text{N}$ -tracer studies. The method described has been used for three years in studies of the transformations, movement, and plant uptake of anhydrous ammonia-derived N during corn production in Iowa. In these studies, small plots ( $^{15}\text{N}$  plots) were located within larger plots (yield plots) that were fertilized by using a conventional applicator.



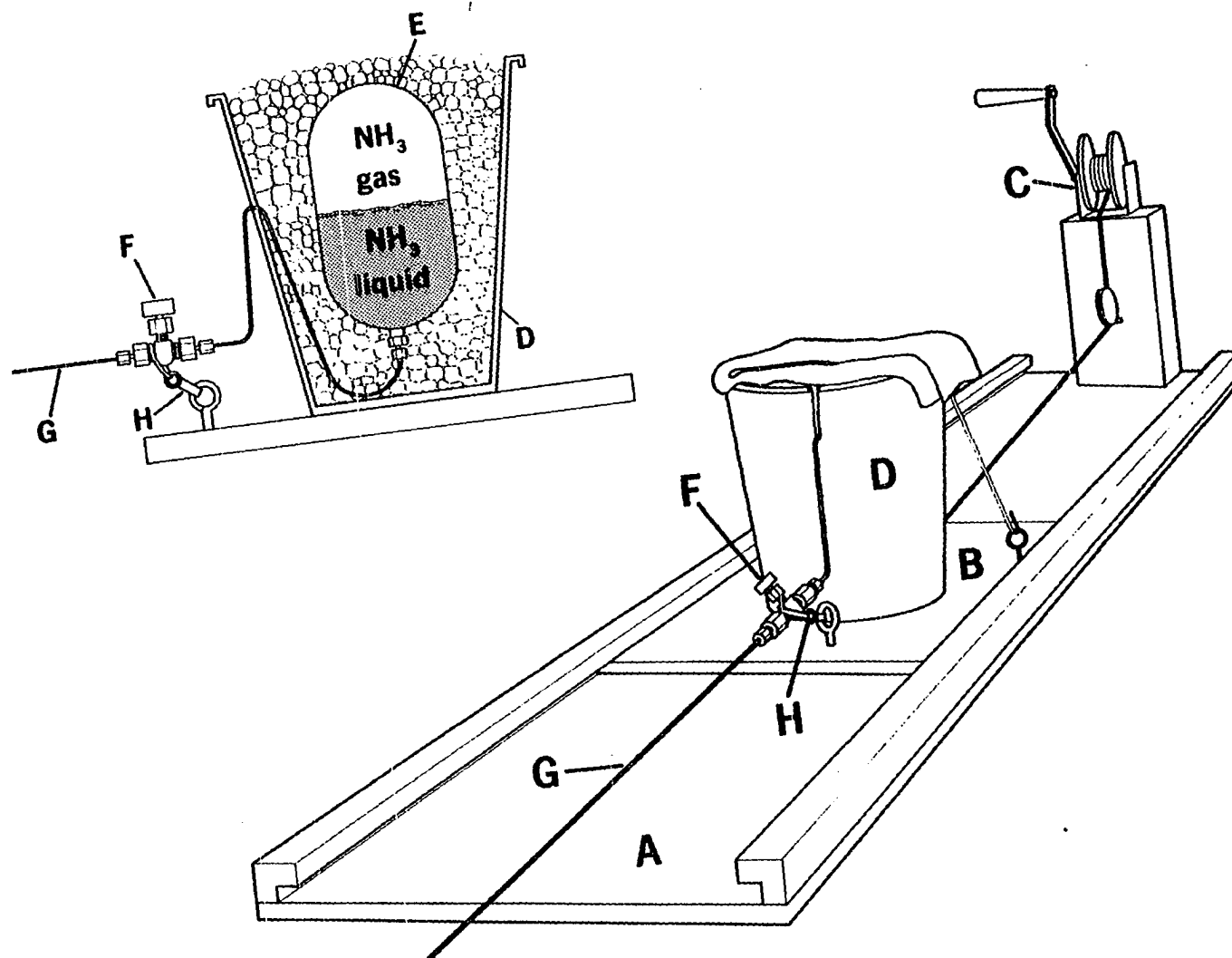
## MATERIALS AND METHODS

Figure 1 illustrates the apparatus for applying mixtures of anhydrous ammonia and nitrification inhibitor to small plots. The procedure for filling a cylinder with a mixture of anhydrous ammonia and nitrification inhibitor is as follows (refer to Fig. 1):

1. With the capillary tube (G) disconnected from the valve (F) on the cylinder, this valve is attached to a vacuum pump, and the cylinder is evacuated to less than 1 kPa.
2. If nitrification inhibitor is to be added, the appropriate amount is placed in a small beaker, the end of the valve on the cylinder is placed in this liquid, and the valve is opened to draw the liquid into the cylinder.
3. The cylinder is attached to a supply of ammonia by means of a stainless-steel capillary tube (1.59 mm OD, 0.51 mm ID, 1 m length). The cylinder is then placed on a top-loading balance, with the capillary tube positioned in such a way as to provide minimum disruption of weighing, and ammonia is permitted to flow from the source to the cylinder. The rate of transfer is maintained at about  $0.2 \text{ g sec}^{-1}$  by manipulating the temperatures of the source and cylinder.
4. When different mixing ratios of ammonia and nitrification inhibitor are required, steps 1 through 3 are repeated using different cylinders.

The procedure for applying mixtures of  $^{15}\text{N}$ -labeled anhydrous

Figure 1. Schematic diagram of the apparatus for application of  $^{15}\text{N}$ -labeled anhydrous ammonia to small plots. The applicator includes: a base, A, which consists of plywood (1.9 by 43 by 305 cm) with grooved sides; a plywood platform, B, that slides on the base; a winch, C, with steel cable to pull the platform; a container, D, filled with water and ice; a 1-L cylinder (Matheson Gas Products, Model 8HD 1000), E, containing  $^{15}\text{N}$ -labeled anhydrous ammonia; a stainless-steel valve (Matheson Model 3712), F, which is attached to the cylinder by an 0.32-cm (OD) stainless-steel tube and Swagelok fittings; a stainless-steel capillary tube (1.59 mm OD, 102 mm ID, 2.6 m length, Alltech Associates), G, that is attached to the valve by a Swagelok fitting, an anchor with cord, H, used to hold the valve in position



ammonia and nitrification inhibitor is as follows:

1. A conventional ammonia applicator that is carefully calibrated for total quantity of N applied per unit area and for uniformity of distribution among outlets on injection knives is used to apply unlabeled anhydrous ammonia to the area surrounding the  $^{15}\text{N}$  plots (i.e., the yield plots).
2. The conventional applicator is then used to place nylon strings into the soil where the bands of labeled ammonia are to be placed in the  $^{15}\text{N}$  plots. This is accomplished by attaching strings to the ammonia outlets, passing the knives through the  $^{15}\text{N}$  plots (without injection of ammonia), and detaching the strings from the ammonia outlets.
3. A stainless-steel capillary tube (G) is placed in the soil where a band of ammonia is to be injected. This is accomplished by attaching a Swagelok union to the capillary tube at the end that connects to the valve on the ammonia cylinder, attaching a string (one end of a string placed in the soil in step 2) to this union, and then pulling it through the soil. Although the stainless-steel capillary tube is flexible and can be pulled around corners, this step is facilitated by removal of small volumes of soil outside each edge of the plot. The amounts of soil removed are only enough to permit pulling the string (or capillary tube) at an angle of about  $45^\circ$  from horizontal.
4. The Swagelok union is washed and removed in a way that assures that particles of soil do not enter the capillary tube, and the

capillary tube is attached to the valve on the ammonia cylinder.

5. The valve on the cylinder is opened so that anhydrous ammonia flows through the capillary tube. The handle (C) on the winch is turned at an appropriate rate so that the capillary tube is slowly pulled through the soil and ammonia is deposited as an even band as the end of the tube advances.
6. After the valve on the cylinder is closed when each band is applied, the capillary tube is detached from the cylinder, the cylinder is weighed (to the nearest 0.1 g on an electronic balance powered by a small generator) and the amount of ammonia applied is determined.

## RESULTS AND DISCUSSION

The rate at which ammonia flows through the capillary tube (G in Fig. 1) during application is determined by the total gas pressure within the cylinder, which is determined by the temperature of the ammonia within the cylinder. Because air is removed from the cylinders before they are filled with ammonia, the total gas pressure in each cylinder is independent of volume of ammonia in the cylinder (until empty). Repeated tests have shown that a constant rate of ammonia flow is achieved within a few seconds if the temperature of the cylinders is constant. To provide constant temperatures, cylinders are maintained in ice-water baths for at least one hour before use and are protected from direct sunlight.

The rate at which ammonia is applied to the soil is determined by the velocity at which the end of the capillary tube is advanced through the soil (i.e., by the speed at which the handle on the winch is rotated). With a minimum amount of calibration and practice (using unlabeled ammonia outside the  $^{15}\text{N}$  plots), an operator can learn to rotate the handle of the winch at an appropriate speed by using only a stopwatch and scale marked on the base of the applicator. Experience has shown that recalibration is required for each day and each set of soil conditions because the flow rate of ammonia is influenced by the condition of the capillary tube and by soil moisture content, which influences the ability of the soil to transfer heat. Although I have found that the rate of ammonia flow through the capillary tube slowly

drifts with time, this presents little problem because the tanks are weighed after application of each band, and it is possible to make minor adjustments in the velocity of the capillary tube to compensate for this drift. This frequent weighing provides exact information concerning the amounts of ammonia applied and also enables early detection of any damage to the capillary tube.

It is necessary to withdraw liquid ammonia from the bottom of the cylinder rather than ammonia gas from the top of the cylinder to assure that no separation of ammonia and nitrification inhibitor occurs within the cylinder. Such a separation could be expected if ammonia were removed from the gaseous phase within the cylinder. Once the mixture of ammonia and nitrification inhibitor enters the capillary tube, this separation is not a problem because the nitrification inhibitor is carried by mass flow even when vaporization of ammonia occurs. Although only one nitrification inhibitor (nitrapyrin) was tested, the method should work with any inhibitor that is soluble in anhydrous ammonia.

It was found that, when applying N at a rate of  $112 \text{ kg ha}^{-1}$  in bands 76 cm apart, about two minutes is required for actual delivery of anhydrous ammonia for a single band 2 m in length. This slow rate of application is essential to assure that a constant rate of flow is established within the first few centimeters of the band. Evidence that a constant rate of ammonia flow is established was obtained by measuring amounts of ammonia dispensed as a function of time. About one half hour is the total time required (includes pulling the capillary tube through the soil, weighing the ammonia cylinder, etc.) to fertilize a three-band

plot. The cost of the time required for application of anhydrous ammonia by this method is excessive by conventional standards, but small compared with the cost of the  $^{15}\text{N}$ -labeled fertilizers applied and the benefits of having mixtures of anhydrous ammonia and nitrification inhibitor evenly injected in bands at a known rate. Repeated tests have shown that this method can be used to apply anhydrous ammonia to small plots with good precision (C.V. of 3% or less).

It is impossible to quantitatively describe the accuracy with which this method reproduces the distribution of fertilizer found when ammonia is applied by conventional methods. However, intensive studies (see Part V) showed that the distributions of ammonia-derived N resulting from this method are similar to distributions reported by other researchers (Blue and Eno, 1954; McIntosh and Frederick, 1958; Hogg and Henry, 1980) who used conventional methods of application. Evidence that the method described adequately reproduces conventional methods also was provided by comparisons of the N contents of corn tissues (leaves at silking, grain and stover at maturity) from  $^{15}\text{N}$  plots and the surrounding yield plots. Such comparisons showed that method of application resulted in no significant differences in response of corn to fertilizer or nitrification inhibitor.



## SUMMARY

A method is described that permits precise application of anhydrous ammonia in bands to plots of the size often used in  $^{15}\text{N}$ -tracer studies. This method involves placing a stainless-steel capillary tube in the soil where the ammonia is to be banded, attaching this tube to a cylinder of ammonia, and then pulling the tube through the soil with deposition of ammonia as an even band. The procedure has marked advantages over previously described methods because it can be used with mixtures of anhydrous ammonia and nitrification inhibitors and because the soil environment at the point of application is representative of the soil environment found when a conventional applicator is used.

PART II. ASSESSMENT OF ERRORS ASSOCIATED WITH LATERAL MOVEMENT OF  $^{15}\text{N}$   
WHEN STUDYING FERTILIZER RECOVERY UNDER FIELD CONDITIONS

## INTRODUCTION

Lateral movement of  $^{15}\text{N}$  is a potential source of error in field studies to determine recovery of fertilizer N by crops, whether this movement occurs by mass flow or diffusion in soils or by translocation in plant tissues. Problems associated with lateral movement are especially important in  $^{15}\text{N}$ -tracer studies because the high cost of labeled fertilizers encourages use of the smallest possible plot size and because plot size requirements are determined largely by the amount of lateral movement that occurs.

One method that has been used (Carter et al., 1967; Malhi and Nyborg, 1983; Power and Legg, 1984;) to eliminate problems associated with lateral movement of labeled N is to place barriers in the soil to confine the plots. Although these barriers eliminate problems associated with lateral movement, they may introduce artifacts that affect fertilizer recovery by crops. These artifacts may result from an inability to perform normal tillage practices, inability of root systems to achieve normal shape or size, creation of artificial pores that may increase aeration or movement of water and solutes, and disruption of macropore systems (Thomas and Phillips, 1979; Bevin and Germann, 1982; White, 1985) that may influence aeration or movement of water and solutes.

Some workers (Bigeriego et al., 1979; Kitur et al., 1984; Meisinger et al., 1985) have alleviated problems associated with lateral movement of labeled fertilizers in field studies by using  $^{15}\text{N}$ -depleted fertilizer

materials, which are less expensive than  $^{15}\text{N}$ -enriched fertilizer materials and, therefore, enable use of relatively large plots. However, it still is necessary to know how small a plot must be before lateral movement introduces significant errors, and this obviously varies with soil and environmental factors. Another major shortcoming of this practice is that, especially in soils having high organic matter contents,  $^{15}\text{N}$ -depleted materials cannot be used to monitor the transformations and movement of fertilizer N within the soil. Therefore, this practice cannot be used in integrated studies of the transformations of fertilizer N in soils and plant responses to this N.

Research for this dissertation involved the initiation of integrated studies of the transformations and movement of  $^{15}\text{N}$ -labeled fertilizers in soils and corn responses to this N during the first and subsequent crops following fertilization. However, I found little information to indicate the size of plots that would be required for these studies. Olson (1980a) reported that reliable measurements of fertilizer uptake could be obtained during the year of fertilization by sampling plants from the centers of plots that were 213 by 214 cm. His results indicate that plots 72 cm in length were not adequate but his data cannot be used to assess the adequacy of plots having lengths between 72 and 214 cm. Furthermore, Olson (1980) suggested that larger plots may be required to study the residual effects of fertilizer N, but he did not speculate on the size of the plots required for residual studies.

My approach to this problem was to use plots that were at least 4.5

m<sup>2</sup> and collect plant tissue samples at various locations inside and outside the <sup>15</sup>N plots to assess the importance of lateral movement of <sup>15</sup>N. The rationale was that such measurements (i) would either provide evidence to show that this plot size was adequate or provide a basis on which I could correct recoveries for lateral movement when lateral movement was a problem, (ii) would be a relatively inexpensive way to insure that lateral movement of labeled N did not invalidate recovery data collected in this study, and (iii) would provide a rational basis for selection of plot sizes for similar <sup>15</sup>N-tracer studies in the future. Reported here is an analysis of the importance of lateral movement of <sup>15</sup>N in these studies and estimates of plot sizes required for future studies.

## THEORY

Lateral movement of N should be detectable by performing isotope ratio analyses on tissues of plants growing near a border between two adjacent plots that are fertilized at a common rate if one of these plots is fertilized with  $^{15}\text{N}$ -labeled fertilizer and the other is fertilized with unlabeled fertilizer. This movement should be detectable whether it occurs by diffusion, by mass flow, or by nutrient translocation in plant roots.

A corn plant positioned exactly on the border between the plots should take half of its N from the plot having labeled fertilizer and half from the plot having unlabeled fertilizer. This plant should have an  $^{15}\text{N}$  enrichment halfway between that of a plant located an infinite distance from the  $^{15}\text{N}$  plot and a plant in the center of an infinitely large  $^{15}\text{N}$  plot. When two plants, one in the plot having labeled fertilizer and one in the plot having unlabeled fertilizer, are located equal distances from the border between the plots, the quantity of unlabeled fertilizer taken up by the plant in the labeled plot should be equal to the quantity of labeled fertilizer taken up by the plant growing in the unlabeled plot.

In the absence of lateral movement of fertilizer by mass flow, curves indicating isotope enrichment of plant tissue as a function of plant position should be symmetrical about the border as shown by the example presented in Figure 1. Under such conditions, area A must equal area a, and area B must equal area b. The sum of areas a and b should

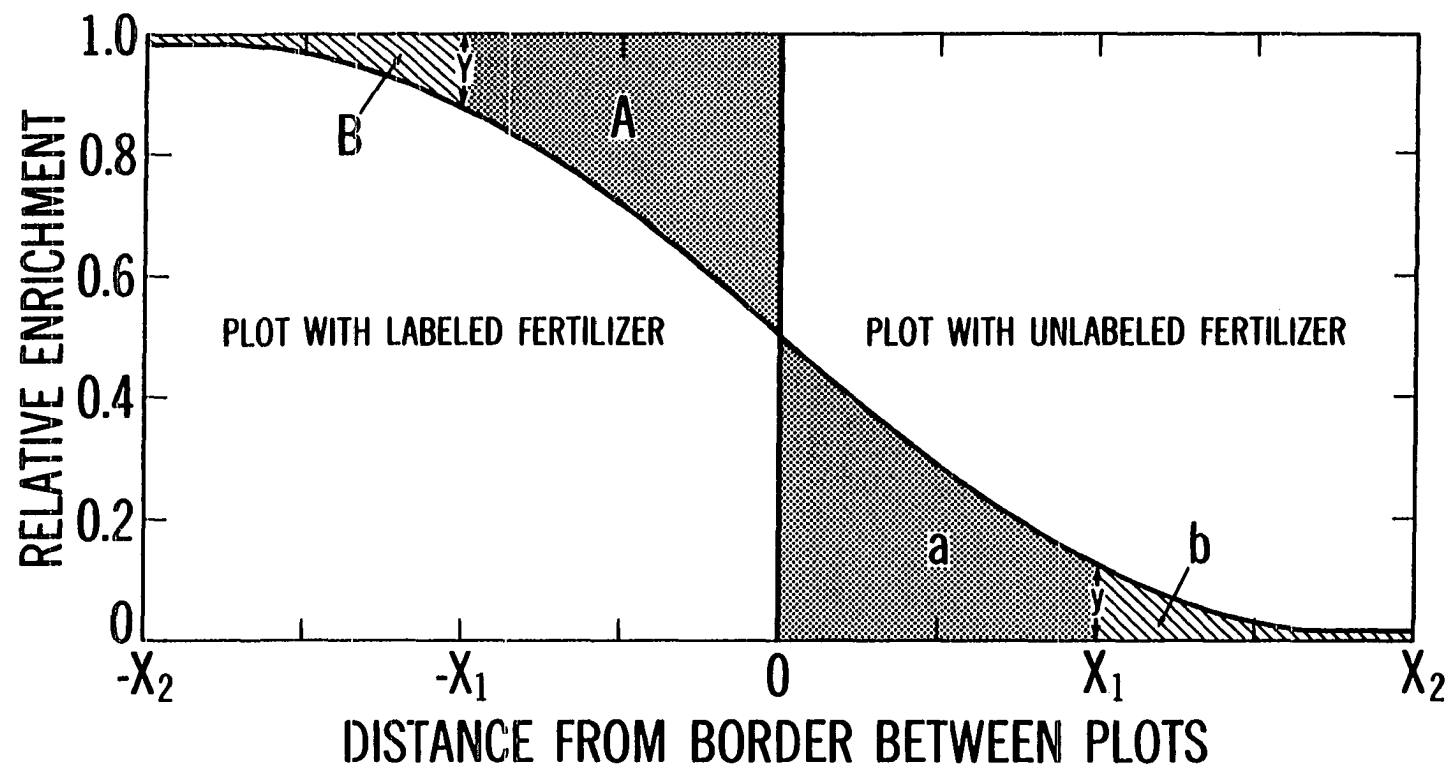


Figure 1. A relationship between relative  $^{15}\text{N}$  enrichment of plant tissue and location of plants near the border between plots having labeled and unlabeled fertilizer

be proportional to the mass of N that moved from the labeled plot and must equal the sum of areas A and B. A plot length of  $2X$  is adequate only if area a is much greater than area b (or area A is much greater than area B).

If the curve is symmetrical about the border, as is illustrated in Figure 1, then the height Y (located at distance  $-X$ ) must equal the height y (located at distance  $X$ ). Therefore, it should be possible to (1) measure the isotopic composition of plant tissue outside the labeled plot and use these measurements to predict the isotopic composition of plants at various locations inside the  $^{15}\text{N}$  plot, (2) predict the plot sizes needed to achieve various levels of accuracy in determinations of fertilizer recovery, and (3) use these measurements to predict the isotopic composition that should be expected in plant tissue on plots sufficiently large that lateral movement of fertilizer N could not affect the isotopic composition of plants located in the center of the plots. Comparison of predicted and observed isotopic composition for plants in the centers of the labeled plots should provide another method to evaluate the adequacy of plot size.

If lateral movement of N occurs by mass flow, especially when marked dispersion accompanies this mass flow, lateral movement may not be detectable by measuring the isotopic composition of plants only within the labeled plots. Also curves indicating isotope enrichment of plant tissue as a function of position need not be symmetrical about the border. When the isotopic compositions of plants inside and outside the labeled plot are measured, a lack of symmetry about the border is



evidence that lateral movement occurred by mass flow or that the  $^{15}\text{N}$  plots were too small.

## MATERIALS AND METHODS

Studies were conducted on an area mapped as Webster (fine-loamy, mixed, mesic, Typic Haplaquolls) and Nicollet (fine-loamy, mixed, mesic, Aquic Hapludolls) soils and located at the Agronomy and Agricultural Engineering Research Center near Ames, Iowa. The experimental design consisted of "main plots" (102 m<sup>2</sup>) and "<sup>15</sup>N subplots" (at least 2.3 x 2 m) within each main plot. <sup>15</sup>N-labeled fertilizer was substituted for unlabeled fertilizer on different subplots each year. Except for location of the <sup>15</sup>N subplots, each main plot received the same N treatment each year.

Forms of N applied were anhydrous ammonia and UAN (urea-ammonium nitrate solution). The anhydrous ammonia was spring-applied in 1982, 1983, and 1984 at rates of 112 and 224 kg N ha<sup>-1</sup> to plots managed by conventional tillage (moldboard plowed in the fall and disked in the spring). Anhydrous ammonia was applied to the main plots by using a conventional applicator and to the <sup>15</sup>N plots by the method described in Part I. The UAN was applied at a rate of 224 kg N ha<sup>-1</sup> to plots managed by conventional and no-tillage systems for 1982 and 1983. It was either surface-applied in the fall before any primary tillage or applied as a band 15 cm below the soil surface (deep-banded) in the spring just before planting. All fertilizer bands were placed midway between corn rows. The UAN was applied to the main plots by using conventional applicators and to subplots by using a syringe (fertilizer injected at 5-cm increments to simulate a band) or a hand sprayer.

In 1982, grain samples from mature corn plants were collected at various positions relative to the  $^{15}\text{N}$  subplot. As shown in Figure 2, some of these samples were collected along lines that were perpendicular to corn rows and some were collected at various distances along corn rows passing through the center of the plots. Similar sets of samples were collected in 1983 from  $^{15}\text{N}$  plots fertilized in 1982 and 1983. For selected plots in 1983 and 1984, all plants were individually sampled along a row extending from the center of the  $^{15}\text{N}$  plot to 2 m outside the  $^{15}\text{N}$  plot.

All grain samples were dried and then ground in a hammer mill. Kjeldahl N was determined on these samples by using the permanganate-reduced iron method to include nitrate (Bremner and Mulvaney, 1982). Isotope ratios were determined by using sodium hypobromite to oxidize ammonium to  $\text{N}_2$  (Hauck, 1982) and by injecting the resulting  $\text{N}_2$  into a Finnigan MAT 250 mass spectrometer.

The fractions (F) of grain N derived from labeled fertilizer were calculated by using equation 1,

$$F = (A_s - A_r) / (A_f - A_r) \quad (1)$$

where  $A_s$ ,  $A_f$ , and  $A_r$  represent the atom percentages  $^{15}\text{N}$  of grain sample, fertilizer applied, and reference grain samples, respectively.

Reference grain samples were collected more than 30 m from  $^{15}\text{N}$  plots.


The relative fractions (Y) of grain N from labeled fertilizer collected at various positions about the border between plots having labeled and unlabeled fertilizer were calculated by equation 2,

$$Y = F_x / F_c \quad (2)$$

```

xxxxxxxxxxxxxxxxxxxxCxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxBxxxxxxxxxxxxxxxxxxxx
xxxGxxxExxDxxAxxDxxExxFxxxGxxx
xxxxxxxxxxxxxxxxxxxxAxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxBxxxxxxxxxxxxxxxxxxxx
xxxxxxxxxxxxxxxxxxxxCxxxxxxxxxxxxxxxxxxxx

```

where  =  $^{15}\text{N}$  subplot

x=unsampled corn plant

A=plants sampled in the center of  $^{15}\text{N}$  plot

B=plants from rows along borders between  
labeled and unlabeled plot

C=plants sampled two corn rows from  $^{15}\text{N}$  plot

D=plants sampled about 38 cm inside  $^{15}\text{N}$  plot

E=plants sampled about 38 cm outside  $^{15}\text{N}$  plot

F=plants sampled about 114 cm outside  $^{15}\text{N}$  plot

G=plants sampled about 190 cm outside  $^{15}\text{N}$  plot

Figure 2. Location of plant samples collected at various positions relative to the  $^{15}\text{N}$  subplot

where  $F_x$  is the fraction of labeled fertilizer from grain samples collected at distance  $x$  from the border and  $F_c$  is the fraction of labeled fertilizer in the grain samples collected in the center of the  $^{15}\text{N}$  plot. The values for  $x$  were assigned so that distances within the labeled plots had negative values.

Equation 3,

$$Y = 1/(1 + e^{px}) \quad (3)$$

was fit to the  $Y$  values within  $N$  treatments. In this equation,  $x$  is the distance from the border between plots and  $p$  is a parameter obtained by the method of least squares. This function has the properties of symmetry illustrated in Figure 1.

Using an iterative process, values for  $Y$  were adjusted to account for lateral movement from the center of the labeled plot. The first step in the iterative process is shown in equation 4,

$$Y'_c = Y_c - 0.01 = 0.99 \quad (4)$$

where  $Y_c$  is the  $Y$  value for the center of the plot. The second step was to adjust all other  $Y$  values by using equation 5.

$$Y'_x = Y'_c Y_x \quad (5)$$

The third step was to fit equation 3 to the  $Y'_x$  values generated by step two and calculate new interim values  $p$  and  $Y'$ . From the interim value for  $Y'_c$ , the next interim values were calculated by using equation 5. The third step was repeated until the difference between  $Y'_c$  of one

iteration and that of the next iteration was less than 0.01. At this point, the values for  $Y'$  were designated as  $Z$  values, the adjusted relative fraction of grain N from labeled fertilizer. Values for  $Z$  represent points on the curve in Figure 1. They represent an approximation of  $F_x/F'_c$ , where  $F'_c$  is the fraction of N from labeled fertilizer expected when plots are sufficiently large that lateral movement did not influence  $F_c$ . An estimation of  $F'_c$  is obtained by equation 6.

$$F'_c = F_c/Z_c \quad (6)$$

When the results of the field studies were plotted as illustrated in Figure 1, the areas corresponding to the area a plus b in Figure 1 were calculated by using equation 7.

$$\text{Area } a+b = \int_0^{\infty} \frac{dx}{1+e^{px}} \quad (7)$$

The areas corresponding to the area b (which is equal to B) in Figure 1 were calculated by equation 8.

$$\text{Area } b = \int_0^{\infty} \frac{dx}{1+e^{px}} \quad (8)$$

The ratio of these two areas ( $b/a+b$ ) is calculated by equation 9,

and is used as an index of model error caused by finite plot size.

$$\frac{b}{a+b} = \int_1^{\infty} \frac{dx}{1+e^{px}} / \int_0^{\infty} \frac{dx}{1+e^{px}} \quad (9)$$

## RESULTS AND DISCUSSION

Table 1 shows mean Y values (relative fractions of grain N from labeled fertilizer) found for corn samples collected at various positions about the borders between plots having labeled and unlabeled fertilizers. Results are presented for the first and second crops following fertilization and for two methods of fertilizer application. The data are presented as means across fertilizers, tillage systems, and years of fertilization because these factors had little effect on Y values. Relative values (i.e., Y values), rather than absolute values (i.e., F values) were used, because the relative values are influenced less by factors other than lateral movement. Even when no lateral movement occurs, losses of fertilizer N by denitrification, ammonia volatilization, or leaching vary among treatments and influence F values.

Lateral movement in a direction perpendicular to the corn rows can be assessed from the B (from rows along borders between labeled and unlabeled plots) and C (from the first rows outside the plots) samples as designated in Figure 2. The mean Y values (see Table 1) for the B samples ranged from 0.43 to 0.49. These values should be 0.50 if the corn plants were positioned exactly on the border between the plots having labeled and unlabeled fertilizers. The small deviations from 0.50 and the high variability of Y values observed in the B samples can be attributed in part to errors in row placement during planting. Although the rows were carefully measured and marked before planting,



Table 1. Mean Y values found for corn grain samples collected at various positions about the borders between plots having labeled and unlabeled fertilizers

Grain sample	Mean Y values <sup>a</sup>	
	Banded N (in spring)	Surface-applied N (in fall)
First crop after fertilization		
A (Center of <sup>15</sup> N plot)	1	1
B (Border row)	0.49 (0.18)	0.47 (0.23)
C (First row outside)	0.01 (0.01)	0.12 (0.21)
D (38 cm inside)	0.82 (0.33)	0.87 (0.18)
E (38 cm outside)	0.19 (0.23)	0.35 (0.18)
F (114 cm outside)	0.01 (0.02)	0.16 (0.10)
G (190 cm outside)	0.00 (0.00)	0.04 (0.04)
Second crop after fertilization		
A (Center of <sup>15</sup> N plot)	1	1
B (Border row)	0.43 (0.17)	0.43 (0.14)
C (First row outside)	0.09 (0.04)	0.10 (0.09)
D (38 cm inside)	0.92 (0.31)	0.81 (0.13)
E (38 cm outside)	0.40 (0.21)	0.68 (0.32)
F (114 cm outside)	0.16 (0.11)	0.39 (0.17)
G (190 cm outside)	0.05 (0.04)	0.13 (0.18)

<sup>a</sup>Values in parentheses show the standard deviation about the mean.

normal flexibility of the hitch between the tractor and the planter allows several centimeters of movement that results in inexact row placement.

The mean Y values for the C samples indicate that lateral movement was detected under some conditions. When the fertilizer was banded in the spring, negligible amounts of lateral movement were detected in the first crop following fertilization. This observation is consistent with the findings of Johnson and Kurtz (1974). However, C samples collected from the second crop following fertilization showed that some lateral movement of fertilizer N occurred. I suspect that this lateral movement was largely due to translocation of N by plants and return of plant residues to the soil. Even in absence of lateral movement of fertilizer N by mass flow or diffusion in the soil, a plant growing in close proximity to the  $^{15}\text{N}$  plot will take up some labeled fertilizer. At the end of the growing season, some of this labeled fertilizer will be deposited as plant material outside the plot. Such a lateral movement by way of plants would be detectable in successive crops.

When UAN was surface applied in the fall, the Y values for C samples indicate that lateral movement of labeled fertilizer was detected in the first as well as the second crop. It is suspected that mass flow or diffusion of fertilizer N in soils may have been important in causing the lateral movement detected in the first crop. As often occurs in Iowa, the soil used in our study was saturated or nearly saturated with water during much of the fall-to-spring period. Mass flow and diffusion of urea and nitrate, which are mobile in soils,

should be expected during such periods. Because work reported in Part IV indicates that most of the fertilizer N recovered in the soil at one year after fertilization was not present as nitrate, probably lateral movement by way of plants was the major factor accounting for additional lateral movement of N detected in the second crop.

Lateral movement in a direction parallel to corn rows can be assessed from the D (38 cm inside the border), E (38 cm outside the border), F (114 cm outside the border), and G (190 cm outside the border) samples as designated in Figure 2. The Y values for these samples are shown in Table 1. Figures 3 and 4 present these data after they have been adjusted relative to  $F'_c$  (the value for  $F_c$  expected when plots are sufficiently large that lateral movement is not a problem). The data presented in Table 1 and Figures 3 and 4 indicate that some lateral movement of fertilizer N occurred along the corn rows.

Table 2 shows parameter values derived from the model used to make Figures 3 and 4. The data presented in this table can be used to evaluate the sufficiency of plot size. One way to evaluate the sufficiency of plot size is to compare values for  $F_c$  and  $F'_c$  shown in Table 2. When these values are similar, the model suggests that plot size was sufficient. Under such conditions, the curves in Figures 3 and 4 have a value of nearly 1.0 at a distance of -1 m, which represents the center of these plots.

A second way to evaluate the sufficiency of plot size is to compare the length of the plot used to the length of the plot required to obtain a specified minimum value for  $Z_c$  (i.e., ratio of  $F_c/F'_c$ ).

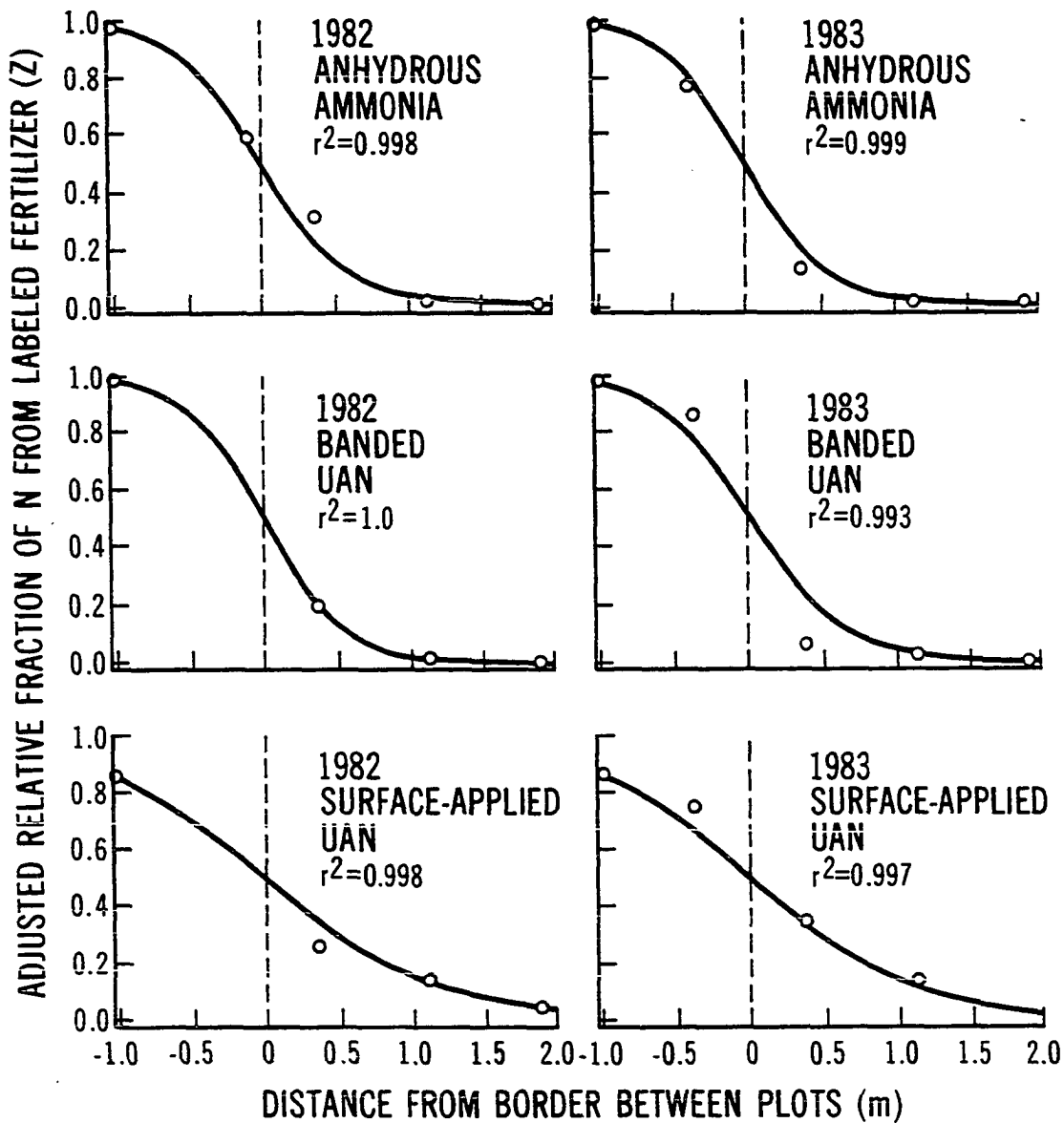


Figure 3. The adjusted relative fractions of grain N from labeled fertilizer at various positions relative to the  $^{15}\text{N}$  subplot for the first crop following fertilization. Circles indicate measured values, solid lines indicate values predicted by the model.

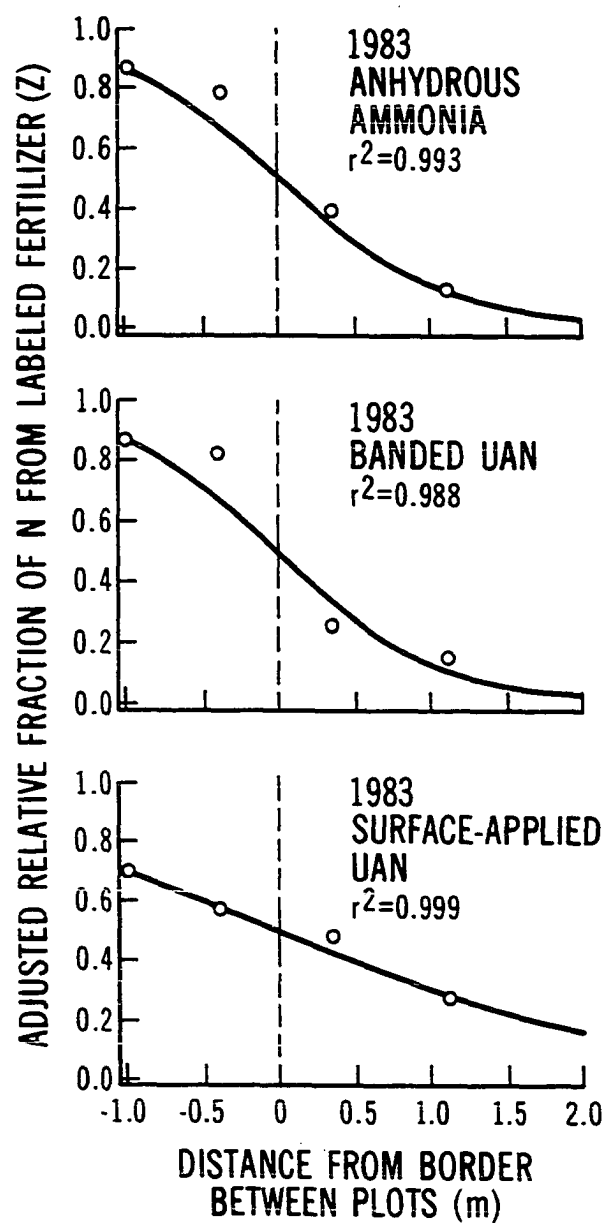


Figure 4. The adjusted relative fractions of grain N from labeled fertilizer at various positions relative to the  $^{15}\text{N}$  plot for the second crop following fertilization

Table 2. Parameters calculated from models describing lateral movement of fertilizer N in plots having various N and tillage treatments

Fertilizer	Year	Tillage	Crop after fertilization
Anhydrous ammonia <sup>a</sup>	1982	Conventional	1
Banded UAN	1982	Conventional	1
Banded UAN	1982	No tillage	1
Surface-applied UAN	1982	Conventional	1
Surface-applied UAN	1982	No tillage	1
Anhydrous ammonia <sup>a</sup>	1983	Conventional	1
Banded UAN	1983	Conventional	1
Banded UAN	1983	No tillage	1
Surface-applied UAN	1983	Conventional	1
Surface-applied UAN	1983	No tillage	1
Anhydrous ammonia <sup>a</sup>	1983	Conventional	2
Banded UAN	1983	Conventional	2
Banded UAN	1983	No tillage	2
Surface-applied UAN	1983	Conventional	2
Surface-applied UAN	1983	No tillage	2

<sup>a</sup>Only data for the 224 kg N ha<sup>-1</sup> rate of anhydrous ammonia is shown.

p	$F_c$	$F'_c$	Required plot length (m)			b/a+b
			$Z_c=0.90$	$Z_c=0.95$	$Z_c=0.99$	
3.39	41	42	1.30	1.74	2.72	0.05
3.74	48	49	1.18	1.58	2.46	0.03
3.39	56	58	1.30	1.74	2.70	0.05
1.69	26	30	2.60	3.48	5.42	0.24
1.74	20	23	2.54	3.40	5.30	0.23
3.55	53	54	1.24	1.66	2.58	0.04
3.00	48	50	1.46	1.96	3.06	0.07
4.10	54	55	1.08	1.44	2.24	0.02
1.70	9	10	2.60	3.48	5.42	0.24
1.87	10	12	2.36	3.18	4.92	0.21
1.78	2	2	2.46	3.30	5.16	0.22
1.51	3	3	2.90	3.88	6.06	0.29
2.44	3	3	1.80	2.42	3.76	0.12
0.61	4	5	7.20	9.64	15.04	0.63
1.09	3	4	4.04	5.42	8.48	0.42

Table 2 shows the requirements for plot length as predicted by the model for specified minimum levels of accuracy. The plot size is probably inadequate if the model indicates need of a plot length substantially larger than was used.

A third way to evaluate the sufficiency of plot size is to consider areas in Figures 3 and 4 that correspond to areas a and b in Figure 1. When area b is much less than the sum of areas a and b, or when  $b/(a+b)$  is very small, plot size is probably sufficient. Consideration of these areas also permits evaluation of validity of the model used to calculate values for  $F'_c$ . When area b represents a significant fraction of area  $(a + b)$ , the assumption that the labeled plot has infinite length is inappropriate because area B must equal area b, and because area B could not exist in a labeled plot having a large length. Under such conditions, values for  $F'_c$  could only be predicted by more complex models. Therefore, the ratio of  $b/(a + b)$  can be used as an index of model reliability (see Table 2). A high ratio indicates a low reliability.

I have a high degree of confidence that a plot size of 2 by 2 m was adequate for the first crop when fertilizers were banded in the spring. I have this confidence because the values for  $F_c$  were within 2% of the values for  $F'_c$ , because the plot size requirement as predicted by these models for an accuracy of  $Z_c > 0.95$  was less than the size of the plots used in these studies, and because the ratio  $b/(a + b)$  ranged from only 0.02 to 0.07.

For practical reasons, I have confidence that this plot size was



adequate for the second crop when fertilizers were applied in the spring. Only 2 to 5% of the N in plants collected from the center of the  $^{15}\text{N}$  plots was from fertilizer applied the previous year. In cases where recoveries are this low, large relative errors have little effect on conclusions concerning amounts of fertilizer recovered and evaluations based on areas a and b have little practical value. Because plot size would have to be substantially increased to significantly improve accuracy and because  $^{15}\text{N}$  represents a major expense in such studies, few studies could justify use of larger plots.

Plots larger than 2 by 2 m may be required when UAN is surface applied in the fall. The plot length requirement as predicted by these models for an accuracy of  $F_c/F'_c > 0.90$  was less than the length (2.3 m) used for this treatment. The values for  $F_c$  were similar to the values of  $F'_c$ . However, the plot size requirements as predicted by these models for an accuracy of  $F_c/F'_c > 0.95$  were larger than 2.8 m and the ratios  $b/(a + b)$  ranged from 0.21 to 0.24. These ratios suggest that the assumptions of these models may not be valid. Because as little as 9% of the N in plants at the center of the  $^{15}\text{N}$  plots was derived from fertilizer in this treatment, there probably is little practical need for greater accuracy in determination of recovery of N by plants.

The isotopic composition of plants collected along 3-m segments of corn rows passing through the centers of  $^{15}\text{N}$  plots from the first, second, and third crops following fertilization (Figure 5 and 6) show that the shapes of the curves predicted by these models (Figures 3 and 4) are reasonable. Data presented in Figure 5 show that the isotopic

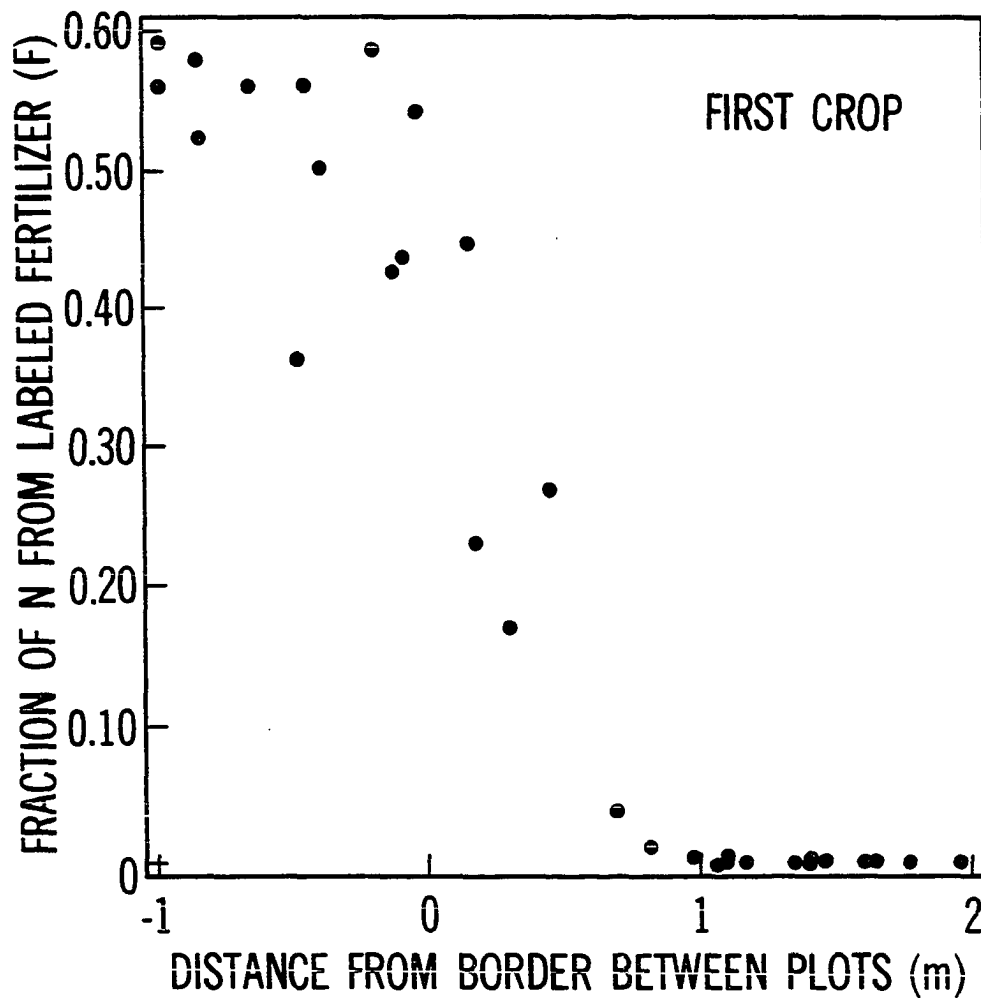


Figure 5. The fraction of N from labeled fertilizer in plants collected along 3-m segments of corn rows passing through the center of  $^{15}\text{N}$  plots for the first crop following fertilization. Data presented were collected in 1983 from selected plots receiving spring-applied anhydrous ammonia

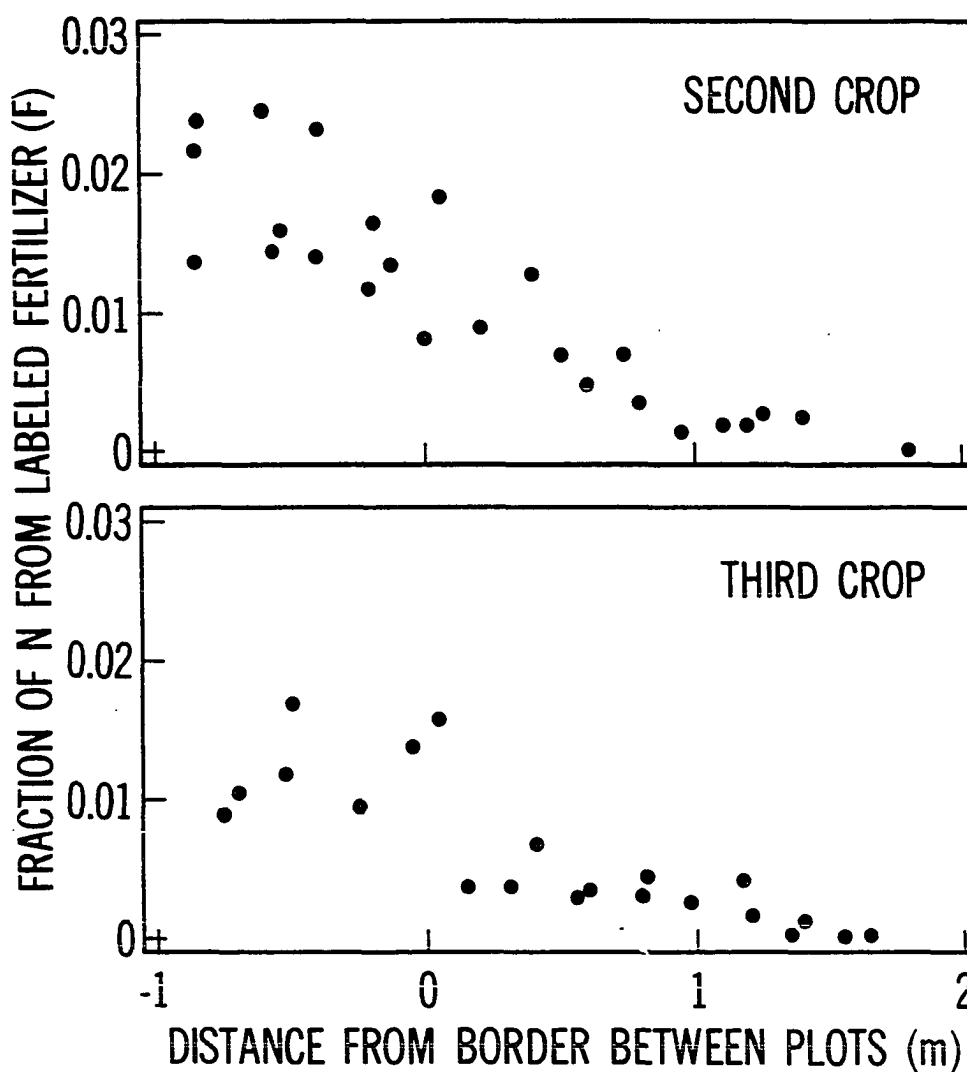


Figure 6. The fraction of N from labeled fertilizer in plants collected along 3-m segments of corn rows passing through the center of  $^{15}\text{N}$  plots for the second and third crops following fertilization. Data presented were collected from selected plots receiving anhydrous ammonia applied in the spring of 1982

composition of plants changed rapidly near the border between plots. This finding provides an explanation for the high variability in Y values shown in Table 1 for the D and E samples because the plants sampled were not located precisely at distances of 38 cm from the border (i.e., the nearest plant to 38 cm was sampled).

The relationships shown in Figures 5 and 6 illustrate the importance of sampling plants outside labeled plots as well as plants inside these plots when assessing errors caused by lateral movement of fertilizer N. The data presented indicate that the isotopic composition of samples collected outside the plot provides much greater sensitivity for detecting lateral movement than does the isotopic composition of samples collected inside the plot. If the plot size used in a study is much smaller than needed, this problem could be detected only by sampling plants outside the plot. For example, only sampling plants from within the labeled plots would have provided no evidence for lateral movement in the second and third crops following fertilization (Figure 6) even though substantial amounts of lateral movement occurred.

The results of these studies indicate that  $^{15}\text{N}$  plots having a size of 2 by 2 m are adequate in size for determining recovery of fertilizer N for corn crops under most conditions. Plots of this size may not be adequate where soils remain saturated for extended periods of time and significant lateral movement of water occurs. When lateral movement of fertilizer N in soils is suspected to be a potential problem, I recommend collection of a few plant samples outside of the  $^{15}\text{N}$  plots to assess the importance of this problem.

## SUMMARY

The high cost of  $^{15}\text{N}$ -labeled fertilizers encourages the use of field plots having minimum size. If plot size is reduced too much, lateral movement of N near the plots by mass flow or diffusion within the soil or by translocation through plant roots can become a significant source of error in determinations of fertilizer N recovery. This study was initiated to assess the importance of lateral movement of labeled fertilizer when unconfined plots are used to determine recovery of fertilizer. Corn grain samples were collected at various positions inside and outside  $^{15}\text{N}$  plots, and the  $^{15}\text{N}$  contents of these samples were determined. The data were fit to mathematical models to estimate the extent to which lateral movement of fertilizer N caused errors in determined values of fertilizer recovery for the first, second, and third crops following fertilization. These models also were used to predict the plot size needed for similar  $^{15}\text{N}$ -tracer studies in the future. The results of these studies indicate that  $^{15}\text{N}$  plots having a size of 2 m by 2 m are sufficiently large for determining recovery of fertilizer N for corn crops under most conditions. Where lateral movement of fertilizer N in soils is suspected to be a problem, I recommend collection of a few plant samples outside of the  $^{15}\text{N}$  plots as insurance against misleading conclusions concerning fertilizer N recovery.

PART III. RESPONSE OF CORN TO <sup>15</sup>N-LABELED ANHYDROUS AMMONIA  
WITH AND WITHOUT NITRAPYRIN

## INTRODUCTION

Nitrification inhibitors are widely recognized as having potential for reducing the amounts of fertilizer N lost by leaching or denitrification (Goring, 1962a, b; Keeney, 1980; Meisinger et al., 1980; Hauck, 1983). However, there is limited published information showing that use of nitrification inhibitors is cost-effective for crop producers in the western portion of the Corn Belt. Hergert and Wiese (1980) reviewed the literature on this topic and concluded that published data from Missouri, Minnesota, Kansas, and Nebraska indicate a rather limited yield response to nitrification inhibitors. Hoeft (1984) reported an average yield increase of -1% for spring-applied nitrification inhibitors in 14 studies conducted at the University of Illinois. The corresponding yield increase was 5% for fall-applied nitrification inhibitors in 12 studies. I found only one published study from Iowa (Gomes, 1982), and this study showed no significant effect of nitrapyrin on corn yields.

Frequent explanations for lack of yield response to nitrification inhibitors are that conditions were not favorable for losses of N by leaching or denitrification (Touchton et al., 1979; Maddux et al., 1985) or that soils had high levels of available N, either from fertilizers or soil organic matter (Guthrie and Bomke, 1980). Blackmer (1986) recently illustrated that the high levels of available N often found in many Corn Belt soils make it very difficult to find experimental conditions where measurable yield responses to nitrification inhibitors could be

expected. Under some conditions, nitrification inhibitors may cause adverse effects on the availability of fertilizers by inducing positional unavailability (Nelson and Huber, 1980; Hoeft, 1984;), immobilization into organic matter (Lewis and Stefanson, 1975; Sochtig and Salfeld, 1977), fixation into clay lattices (Juma and Paul, 1983), or volatilization as ammonia (Bundy and Bremner, 1974).

Because of the large amounts of fertilizer N being used, because of the need to improve the efficiency of this fertilization for economic and environmental reasons, and because nitrification inhibitors seem to offer great potential as tools to improve the efficiency of N fertilization, there is a great need for studies that show why yield responses are seldom observed in response to nitrification inhibitors in the western portion of the Corn Belt.

Reported here are the results from a three-year study that was conducted at two sites in Iowa to evaluate the response of corn (Zea mays L.) to  $^{15}\text{N}$ -labeled anhydrous ammonia with and without nitrapyrin. The rationale for this study was that the use of  $^{15}\text{N}$  tracers would enable collection of sufficient information to explain why yield responses were, or were not, obtained.



## MATERIALS AND METHODS

Plots measuring 16.7 by 6.1 m were established at the Northeast Iowa Research Center near Nashua and at the Agronomy and Agricultural Engineering Research Center near Ames. The plots near Nashua were located on an area mapped as Readlyn (fine-loamy, mixed, mesic Aquic Hapludolls) and the plots near Ames were located on an area mapped as Nicollet (fine-loamy, mixed, mesic Aquic Hapludolls) and Webster (fine-loamy, mixed, mesic, Typic Haplaquolls). The soils in both areas are classified as being somewhat poorly drained. The plots near Ames had been planted to corn in 1980 and 1981, but had received no fertilizer treatments. The plots near Nashua had been planted to soybeans (Glycine max L.) during 1980 and 1981.

Fertilizer treatments applied to the plots were 0, 112, and 224 kg N ha<sup>-1</sup> as spring-applied anhydrous ammonia and 112 and 224 kg N ha<sup>-1</sup> as spring-applied anhydrous ammonia plus nitrapyrin (2.4 L ha<sup>-1</sup> N-Serve 24). All plots were fertilized with P (56 kg ha<sup>-1</sup>) and K (168 kg ha<sup>-1</sup>) that was broadcast and disked into the soil before planting. By using methods described in Part I, anhydrous ammonia having about 4 atom percent <sup>15</sup>N was substituted for unlabeled anhydrous ammonia on small (4.6 m<sup>2</sup>) plots that were located within the larger plots. For clarity, I refer to the small plots as "<sup>15</sup>N plots" and the large plots as "yield plots" in this paper. Because the labeled ammonia was applied at different locations within the yield plots each year, the <sup>15</sup>N plots are further identified by the year in which the labeled ammonia was applied

(see Figure 1). All treatments were replicated three times.

Corn (SAR SX4900, 110 day maturity class) was planted to give a population of about 58,000 plants  $\text{ha}^{-1}$ . Planting dates were June 4 and 5 for 1982, May 6 and 4 for 1983, and May 17 and 18 for 1984 at Ames and Nashua, respectively. Rainfall and class A pan evaporation data were collected during the growing season at each location. Grain yields were measured by using a combine to harvest 12.2 m segments of three rows from each yield plot. All grain yields were adjusted to 15.5% moisture.

When 50% of the silks had emerged, samples of leaf tissue (leaves opposite and below the primary ear) were collected from all plots (yield plots and  $^{15}\text{N}$  plots). At physiological maturity, whole plant samples were collected from all plots and partitioned into grain and stover (including cobs) components. All plant materials were dried at  $65^{\circ}\text{C}$  and ground for analysis. Grinding was done with a hammer mill for grain samples and with a Cyclone mill for leaves and stover. The permanganate-reduced iron modification of the Kjeldahl procedure (Bremner and Mulvaney, 1982) was used to determine N content of subsamples that had been dried for at least 48 hours at  $65^{\circ}\text{C}$ . Isotope ratio analyses were performed by reacting the resulting Kjeldahl distillates with sodium hypobromite in evacuated Rittenberg flasks as described by Hauck (1982) and injecting the resulting dinitrogen gas into a Finnigan MAT 250 mass spectrometer. Statistical calculations followed Snedecor and Cochran (1967) and Steel and Torrie (1960). Critical values for nutrient concentrations in plant tissues were determined using the procedure of Cate and Nelson (1971).

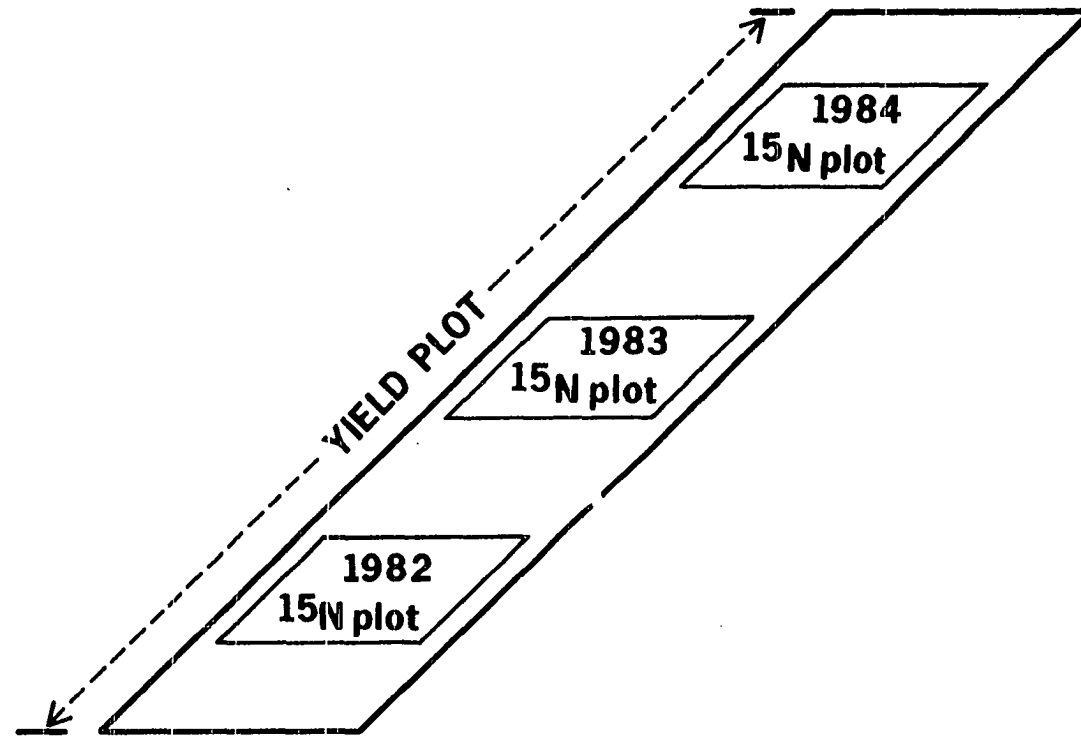


Figure 1. Arrangement of  $^{15}\text{N}$  plots within yield plots

## RESULTS AND DISCUSSION

During the three years these studies were conducted, the amounts of rainfall that occurred during the 2-month period following fertilization often were above normal and pan evaporation was often below normal (Table 1). Therefore, potential for loss of fertilizer N by leaching or denitrification was above normal. Data presented in Part IV show that more than half of the fertilizer N was lost from the rooting zone during the first year by processes other than plant harvest in these studies. In 1982, a period of rainfall delayed fertilization and planting until June 2. The most significant rainfall occurred in June of 1983, when more than 169 mm of precipitation occurred during a 4-day period at the Ames site.

During 1983 and 1984, moisture stress on plants was above average during the grain-filling period. Less than 40 mm of rainfall occurred between 5 July and 21 August at each location in 1983. Less than 15 mm of rainfall occurred between 17 July and 17 August in 1984. This moisture stress resulted in yields that were less than anticipated but generally consistent with county averages.

Corn grain yields ranged from 1.3 to 7.5 Mg ha<sup>-1</sup> during the growing years of 1982, 1983, and 1984 (Table 2). Because nitrapyrin was not applied without anhydrous ammonia, the statistical data presented in Table 2 exclude unfertilized plots. Although the data are not shown in Table 2, a highly significant yield response was obtained with the first increment (112 kg N ha<sup>-1</sup>) of fertilizer at both locations in all years.

Table 1. Precipitation and open-pan evaporation data at Ames and Nashua

Period	Ames				Nashua			
	1982	1983	1984	Normal <sup>a</sup>	1982	1983	1984	Normal <sup>a</sup>
-----mm-----								
<u>Precipitation</u>								
Jan-Mar	133	135	64	100	100	128	133	113
Apr	70	80	173	66	74	63	106	80
May	155	158	129	109	178	261	97	113
June	67	232	167	132	77	178	97	118
July	156	97	86	84	106	85	62	101
Aug	88	107	8	98	89	59	32	103
Sept	48	81	101	84	73	255	68	106
Oct	67	159	92	51	82	72	146	66
Nov-Dec	183	147	95	67	163	140	68	78
Jan-Dec	967	1196	915	790	942	1240	809	878
<u>Open-pan Evaporation</u>								
Jan-Mar	nd	nd	nd	nd	nd	nd	nd	nd
Apr	nd	nd	nd	nd	nd	nd	nd	nd
May	134	160	174	182	119	140	122	162
June	168	206	207	204	170	157	166	186
July	181	228	224	212	183	178	161	195
Aug	152	215	214	173	148	166	162	160
Sept	107	159	167	132	90	119	114	116
Oct	87	100	75	96	74	63	49	85
Nov-Dec	nd	nd	nd	nd	nd	nd	nd	nd

<sup>a</sup>Normal values for precipitation and pan evaporation are long-term averages reported by Shaw and Waite (1964) and Shaw (1981).

Table 2. Yields of corn grain as affected by N rate and nitrification inhibitor

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup>		-----Mg ha <sup>-1</sup> -----								
Control	-	2.50	1.74	2.13	2.12	5.70	2.43	1.33	3.15	2.64
112	-	6.51	4.21	3.71	4.81	6.83	4.54	5.47	5.62	5.21
112	+	6.71	3.94	4.32	4.99	6.96	3.25	5.50	5.24	5.11
224	-	7.27	5.23	5.06	5.85	6.88	4.45	5.63	5.66	5.75
224	+	7.48	5.50	4.90	5.96	7.01	3.93	4.81	5.25	5.60

Statistical data:<sup>a</sup>

N Rate	0.08 <sup>b</sup>	0.02	0.01	0.01	NS	NS	NS	NS	0.01
NI	NS	NS	NS	NS	NS	0.01	NS	0.03	NS
N Rate * NI	NS	NS	NS	NS	NS	0.06	NS	NS	NS
CV (%)	9	14	9	11	6	7	11	9	10
LSD <sup>c</sup> (P=0.05)	--	--	--	--	--	0.4	--	0.3	--

<sup>a</sup>Data presented show the results of ANOVA excluding the control. If the control is included, the response to the first increment of N is highly significant ( $P > 0.01$ ) in all years and at both locations.

<sup>b</sup>NS indicates a probability level  $> 0.10$ .

<sup>c</sup>LSD values apply only to comparisons of means for treatments with and without NI. An LSD value is shown only if the effect of NI or the N rate x NI interaction was significant at the 10% level or less.

The second increment of fertilizer resulted in an additional increase in grain yields at the Ames location each year but it did not result in an additional increase at Nashua in any of the years. A partial explanation for the differences between sites is that the Nashua location had been cropped to soybeans in 1981, whereas the Ames location had been cropped for at least three years to continuous corn without fertilization. As should be expected from this difference in cropping history, there was more inorganic N in the soil at the Nashua location than at the Ames location when the study was initiated (see Part IV).

Of the 6 site-years studied, nitrapyrin had a statistically significant effect on grain yields only in 1983 at Nashua and this effect was to decrease grain yields. There are several possible reasons for lack of response to nitrapyrin (Blackmer, 1986) and several possible ways that nitrapyrin could decrease yields. To more closely study the effects of nitrapyrin, I determined N concentration, dry matter yields and percentage NDFF (nitrogen derived from fertilizer) for various plant tissues from each plot. Tables 3 through 6 present the results of these determinations and statistical analyses of the effects of N rate and nitrapyrin.

When trying to determine why nitrapyrin did not increase yields, it is necessary to identify those situations where nitrapyrin could not have been expected to increase yields. Clearly, nitrapyrin could not be expected to increase yields in any situation where adequate N was present to produce maximum yields without nitrapyrin. To identify these

Table 3. Nitrogen concentration of leaves, grain, and stover as affected by N rate and nitrification inhibitor

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup>		-----g Kg <sup>-1</sup> dry matter-----								
<u>N concentration in corn leaves</u>										
Control	-	11.3	10.5	11.9	11.2	25.6	16.0	13.7	18.4	14.8
112	-	24.9	21.7	24.0	23.5	30.3	21.1	24.7	25.4	24.4
112	+	27.0	21.6	25.5	24.6	29.4	22.4	25.5	25.8	25.2
224	-	27.1	26.3	26.6	26.7	28.8	24.9	26.2	26.7	26.7
224	+	30.5	26.8	27.1	28.1	29.4	27.0	25.8	27.4	27.7

Statistical data:<sup>a</sup>

N Rate	0.01 <sup>b</sup>	0.01	NS	0.01	NS	0.02	NS	0.05	0.01
NI	0.01	NS	NS	0.07	NS	NS	NS	NS	0.06
N Rate * NI	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	4	10	9	8	7	12	3	8	8
LSD <sup>c</sup> (P=0.05)	1.5	--	--	1.4	--	--	--	--	1.0

N concentration in grain

Control	-	11.4	12.9	14.4	12.9	11.9	14.4	12.8	13.0	13.0
112	-	13.5	15.1	18.0	15.5	14.9	15.3	16.4	15.5	15.5
112	+	14.2	15.4	18.0	15.9	15.3	16.7	17.1	16.4	16.1
224	-	15.5	16.5	18.4	16.8	15.3	18.1	18.5	17.2	17.0
224	+	15.2	16.0	19.4	16.9	15.3	18.8	18.9	17.7	17.3

Statistical data:

N Rate	0.01	NS	NS	0.02	NS	0.03	0.03	0.01	0.01
NI	NS	NS	NS	NS	NS	NS	NS	0.09	NS
N Rate * NI	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	5	9	7	8	5	8	6	7	8
LSD (P=0.05)	--	--	--	--	--	--	--	0.8	--

<sup>a</sup>Data presented show the results of ANOVA excluding the control. If the control is included, the response to the first increment of N is highly significant ( $P>0.01$ ) in all years and at both locations.

<sup>b</sup>NS indicates a probability level  $>0.10$ .

<sup>c</sup>LSD values apply only to comparisons of means for treatments with and without NI. An LSD value is shown only if the effect of NI or the N rate x NI interaction was significant at the 10% level or less.



Table 3. Continued

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
<hr/>										
kg N ha <sup>-1</sup>		-----g Kg <sup>-1</sup> dry matter-----								
<hr/>										
N concentration in stover										
Control	-	5.6	4.5	4.8	5.0	5.4	4.7	4.8	5.0	5.0
112	-	5.7	5.7	5.6	5.7	8.4	5.9	5.8	6.7	6.2
112	+	6.4	5.3	5.6	5.8	8.0	7.6	6.5	7.4	6.6
224	-	5.9	7.2	5.9	6.3	8.4	8.0	8.0	8.2	7.2
224	+	7.0	6.9	7.8	7.2	9.2	8.8	7.9	8.5	7.8
Statistical data:										
N Rate		NS	0.01	0.10	0.01	NS	0.01	0.02	0.01	0.01
NI		0.03	NS	NS	0.07	NS	0.02	NS	0.07	0.01
N Rate * NI		NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)		8	11	19	13	11	10	11	10	12
LSD (P=0.05)		0.7	--	--	0.6	--	1.0	--	0.5	0.4

Table 4. The amounts of N in grain and stover as affected by fertilizer N rate and nitrification inhibitor

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup>		-----kg N ha <sup>-1</sup> -----								
<u>Amount of N in stover</u>										
Control	-	36.6	20.7	28.4	28.6	61.0	22.7	31.2	38.3	33.4
112	-	55.2	44.1	40.4	46.6	105.8	31.1	55.9	64.3	55.4
112	+	63.0	47.2	45.9	52.0	85.8	56.1	64.2	68.7	60.3
224	-	61.1	78.4	39.6	59.7	94.6	57.7	89.9	80.7	70.2
224	+	66.3	54.5	58.3	59.7	99.7	64.1	73.0	78.9	69.3

Statistical data:<sup>a</sup>

N Rate	NS <sup>b</sup>	0.01	NS	0.01	NS	0.01	0.10	0.01	0.01
NI	NS	0.01	0.03	NS	NS	0.02	NS	NS	NS
N Rate * NI	NS	0.01	NS	NS	NS	0.10	NS	NS	NS
CV (%)	12	9	16	15	17	16	26	19	18
LSD <sup>c</sup> (P=0.05)	--	7.3	11.3	--	--	12.2	--	--	--

Amount of N in grain

Control	-	23.8	19.3	26.1	23.1	57.0	29.5	22.3	36.3	29.7
112	-	74.2	54.4	59.5	62.7	85.9	58.7	75.9	73.5	68.1
112	+	80.5	49.9	62.2	64.2	89.5	46.0	79.2	71.6	67.9
224	-	95.5	73.0	78.6	82.4	88.6	68.2	85.8	80.9	81.4
224	+	95.7	74.2	80.3	83.4	90.9	62.7	77.0	76.9	80.1

Statistical data:

N Rate	0.02	0.01	0.02	0.01	NS	0.03	NS	0.08	0.01
NI	NS	NS	NS	NS	NS	0.10	NS	NS	NS
N Rate * NI	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	11	14	14	13	7	14	14	13	14
LSD (P=0.05)	--	--	--	--	--	11.3	--	--	--

<sup>a</sup>Data presented show the results of ANOVA excluding the control. If the control is included, the response to the first increment of N is highly significant (P>0.01) in all years and at both locations.

<sup>b</sup>NS indicates a probability level >0.10.

<sup>c</sup>LSD values apply only to comparisons of means for treatments with and without NI. An LSD value is shown only if the effect of NI or the N rate x NI interaction was significant at the 10% level or less.

Table 4. Continued

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup>		-----kg N ha <sup>-1</sup> -----								
<u>Amount of N in whole plant</u>										
Control	-	60.4	40.0	54.4	51.6	118.0	52.2	53.6	74.6	63.1
112	-	129.4	98.5	99.9	109.3	191.7	89.7	131.8	137.8	123.5
112	+	143.5	97.1	108.1	116.2	175.4	102.2	143.4	140.3	128.3
224	-	156.7	151.4	118.2	142.1	183.2	125.9	175.7	159.9	150.5
224	+	162.0	128.7	138.6	143.1	190.6	126.8	150.0	155.8	149.5
Statistical data:										
N Rate		0.02	0.01	0.03	0.01	NS	0.01	0.06	0.01	0.01
NI		NS	0.10	0.10	NS	NS	NS	NS	NS	NS
N Rate * NI		NS	NS	NS	NS	NS	NS	0.10	NS	NS
CV (%)		9	10	12	11	9	8	11	9	11
LSD (P=0.05)		--	16.8	21.5	--	--	--	24.1	--	--

Table 5. Percentage NDFF (N derived from fertilizer) in the leaves, grain and stover as affected by N rate and nitrification inhibitor

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup> ----- % -----										
NDFF in leaves										
112	-	nd	57	46	52	nd	43	59	51	51
112	+	nd	53	46	50	nd	44	52	48	49
224	-	nd	62	56	59	nd	46	57	51	55
224	+	nd	61	55	58	nd	49	58	53	56
Statistical data:										
N Rate		nd	0.01 <sup>a</sup>	NS	0.02	nd	NS	NS	NS	0.01
NI		nd	0.10	NS	NS	nd	NS	NS	NS	NS
N Rate * NI		nd	NS	NS	NS	nd	NS	NS	NS	NS
CV (%)		nd	4	19	12	nd	16	6	11	11
LSD <sup>b</sup> (P=0.05)		nd	3.1	--	--	nd	--	--	--	--
NDFF in grain										
112	-	25	41	40	35	26±	30	49	35	35
112	+	46	38	41	42	28	33	43	35	38
224	-	45	45	48	46	38	45	53	45	46
224	+	45	62	55	54	32	47	54	44	49
Statistical data:										
N Rate		0.01	0.01	0.04	0.01	0.05	0.01	0.01	0.01	0.01
NI		0.01	0.08	NS	0.01	NS	NS	NS	NS	0.01
N Rate * NI		0.01	0.03	NS	NS	NS	NS	0.10	NS	NS
CV (%)		4	13	15	13	18	18	6	13	13
LSD (P=0.05)		2.2	8.3	--	4.0	--	--	4.8	--	2.7

<sup>a</sup>NS indicates a probability level >0.10.

<sup>b</sup>LSD values apply only to comparisons of means for treatments with and without NI. An LSD value is shown only if the effect of NI or the N rate x NI interaction was significant at the 10% level or less.

Table 5. Continued

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup> ----- % -----										
NDFD in stover										
112	-	29	44	40	38	27	30	53	36	37
112	+	43	41	42	42	29	37	46	37	40
224	-	42	43	52	46	39	46	56	46	46
224	+	44	57	56	52	30	44	56	42	47
Statistical data:										
N Rate		0.02	0.10	0.04	0.01	0.10	0.01	0.05	0.01	0.01
NI		0.01	NS	NS	0.02	NS	NS	NS	NS	NS
N Rate * NI		0.03	0.08	NS	NS	NS	NS	NS	NS	NS
CV (%)		10	16	17	14	21	13	8	14	14
LSD (P=0.05)		5.3	10.3	--	4.3	--	--	--	--	--

Table 6. Amounts of fertilizer N in grain and stover as affected by N rate and nitrification inhibitor

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup>		-----kg N ha <sup>-1</sup> -----								
Fertilizer N in stover										
112	-	15.8	19.2	16.4	17.1	28.2	9.2	29.9	22.4	19.8
112	+	27.0	19.4	19.4	21.9	24.6	20.6	29.7	25.0	23.5
224	-	26.6	33.4	20.4	26.8	36.6	26.7	50.5	37.9	31.3
224	+	29.0	31.2	32.9	31.0	29.6	28.4	40.4	32.8	31.9

Statistical data:

N Rate	0.04 <sup>a</sup>	0.01	0.05	0.01	0.04	0.01	0.07	0.01	0.01
NI	0.03	NS	0.06	0.02	0.08	0.05	NS	NS	0.10
N Rate * NI	NS	NS	NS	NS	NS	0.10	NS	NS	NS
CV (%)	17	19	26	22	15	22	29	23	23
LSD <sup>b</sup> (P=0.05)	5.9	--	9.0	3.8	6.2	6.5	--	--	2.9

Fertilizer N in grain

112	-	18.5	22.5	23.9	21.6	22.6	17.5	37.2	25.8	23.7
112	+	36.4	19.1	25.6	27.2	25.1	15.3	33.9	24.8	25.9
224	-	42.9	33.0	37.7	37.9	33.1	31.0	45.7	36.6	37.2
224	+	43.3	45.5	44.4	44.4	29.7	30.0	42.0	33.9	39.2

Statistical data:

N Rate	0.01	0.01	0.01	0.01	0.04	0.01	0.10	0.01	0.01
NI	0.01	NS	NS	0.01	NS	NS	NS	NS	NS
N Rate * NI	0.01	0.10	NS	NS	NS	NS	NS	NS	NS
CV (%)	12	24	22	20	18	24	19	20	20
LSD (P=0.05)	6.2	10.2	--	4.7	--	--	--	--	--

<sup>a</sup>NS indicates a probability level >0.10.<sup>b</sup>LSD values apply only to comparisons of means for treatments with and without NI. An LSD value is shown only if the effect of NI or the N rate x NI interaction was significant at the 10% level or less.

Table 6. Continued

N Rate	NI	Ames				Nashua				Overall mean
		1982	1983	1984	Mean	1982	1983	1984	Mean	
kg N ha <sup>-1</sup>		-----kg N ha <sup>-1</sup> -----								
Fertilizer N in whole plant										
112	-	34.2	41.8	40.3	38.8	50.8	26.7	67.1	48.2	43.5
112	+	63.5	38.5	45.0	49.0	49.7	35.9	63.6	49.7	49.4
224	-	69.5	66.5	58.0	64.7	69.6	57.7	96.1	71.8	68.0
224	+	72.3	76.8	77.3	75.4	59.3	58.4	82.4	66.7	71.1
Statistical data:										
N Rate		0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.01	0.01
NI		0.01	NS	0.10	0.01	NS	NS	NS	NS	0.05
N Rate * NI		0.02	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)		12	20	21	18	12	19	16	15	16
LSD (P=0.05)		9.9	--	18.4	7.3	--	--	--	--	4.5

situations, I determined critical N levels for leaves at silking and grain and stover at harvest (Figure 2) and used these levels to help explain the data presented in Tables 3 through 6. The critical levels shown in Figure 2 are similar to those reported by other workers (Pierre et al., 1977; Jones and Eck, 1973).

Of the 12 rate-site years where fertilizer was applied without nitrapyrin: the N content of the stover was within 5% of, or exceeded, the critical level at 12 rate-site years (Table 3); the N content of the grain was within 5% of, or exceeded, the critical level at 11 rate-site years; and the N content of the leaves at silking was within 5% of, or exceeded, the critical level at 7 rate-site years. The seeming internal inconsistency of N sufficiency level as indicated by the various plant parts can probably be explained by moisture stresses that occurred after leaf samples were collected. Overall, these observations indicate that an abundance of available N must be considered a probable reason why nitrapyrin did not increase grain yields in this study. These observations suggest that selection of the appropriate amounts of N to apply with nitrapyrin may be one of the most difficult problems associated with demonstrating the benefits of using nitrification inhibitors.

Because an abundance of available N made it impossible for nitrapyrin to increase yields by preventing losses of fertilizer N, the best that could be expected from nitrapyrin would be to promote luxury uptake of N (i.e., increase N uptake without increasing grain yields) or to increase the availability of fertilizer-derived N relative to soil-



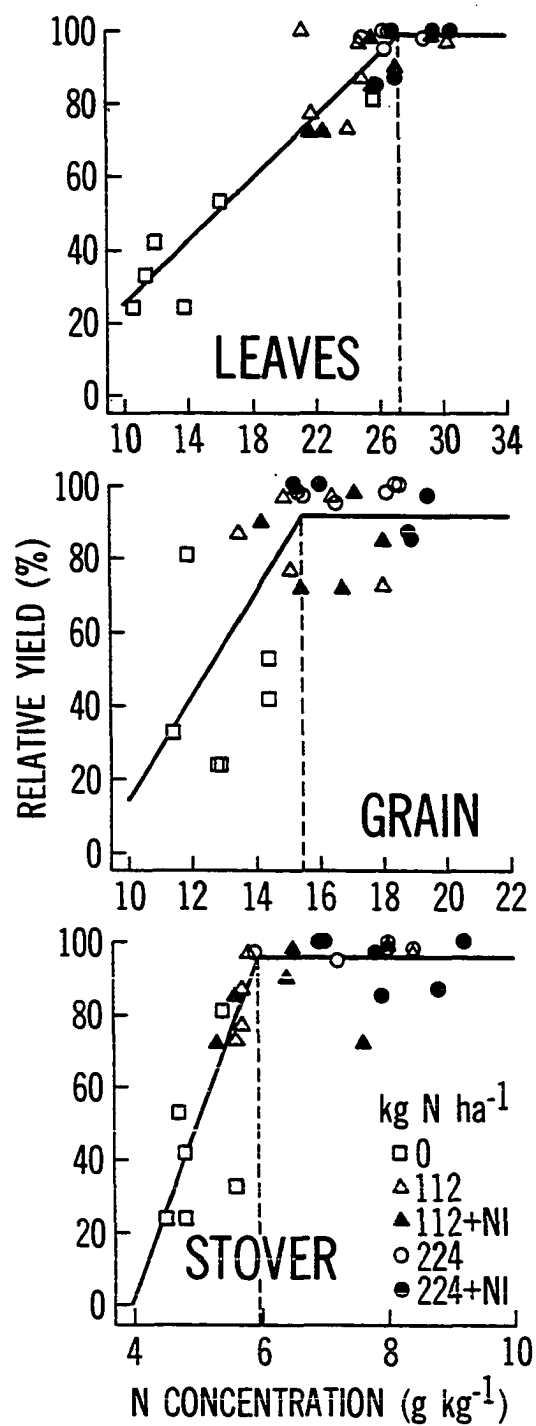


Figure 2. Relationship between relative yields and N concentration in plant tissue. Dashed lines indicate critical values

derived N (i.e., increase percentage NDFF in tissue samples). An overall (years and sites pooled) statistical analysis showed that nitrapyrin had no effect on N uptake (Table 4). Considering the 12 rate-site years individually, nitrapyrin significantly increased N uptake in the stover twice and significantly decreased N uptake in the grain, stover, and total plant once, once, and twice, respectively. An overall (years and sites pooled) statistical analysis showed that nitrapyrin increased the percentage NDFF in the grain but had no effect on percentage NDFF in leaves or stover (Table 5). Considering the 12 rate-site years individually, nitrapyrin significantly increased percentage NDFF twice in grain and twice in the stover. However, it also decreased percentage NDFF in the grain at one rate-site year. These observations suggest that nitrapyrin did not have a clear effect on luxury uptake of N or on the relative availability of fertilizer-derived N across all rate-site years. An individual consideration of each rate-site year helps explain what happened.

There is one rate-site year (when  $112 \text{ kg N ha}^{-1}$  was applied at Nashua in 1982) at which nitrapyrin had no significant effect on any of the characteristics measured and addition of the second increment of fertilizer did not increase grain yields. Nitrapyrin could not be expected to increase yields under these conditions and there is little basis for discussion of the effects of nitrapyrin.

There are, however, three site years (Ames 1982, 1983, and 1984) in which the addition of the second increment of N increased grain yields but addition of nitrapyrin with the first increment did not increase

grain yields. In 1984, nitrapyrin had no significant effects on any of the parameters measured. Because the yield increases were substantial even though they were not statistically significant, it is probable that lack of experimental sensitivity was the major reason for not finding a significant yield increase at this rate-site year. Of the 12 rate-site years considered, I suggest that this one showed the most favorable overall response to nitrapyrin.

In 1983 at Ames, the only significant effect of nitrapyrin was to decrease percentage NDFF in the leaves. This observation indicates that nitrapyrin decreased the availability of fertilizer N early in the season at this rate-site year. In 1982 at Ames, nitrapyrin increased the N contents of leaves and stover, increased percentage NDFF in grain and stover, and increased recovery of fertilizer in grain and stover. However, because nitrapyrin increased the N content of the leaves and stover to above the critical level without increasing yields, it must be concluded that nitrapyrin had some adverse effect on plant growth at this rate-site year even though it increased the availability of fertilizer N. It also must be concluded that, at these site years, additional benefits could not be expected from inhibitors that retard nitrification for longer periods of time.

Adverse effects of nitrapyrin were clearly apparent at Nashua in 1983, where nitrapyrin decreased yields when applied with the first or second increment of N. When applied with the second increment of N at Ames in 1983, nitrapyrin increased percentage NDFF in grain and stover but decreased the amount of N in the above-ground portion of the plants.

When applied with the first increment of N at Nashua in 1984, the only significant effect of nitrapyrin was to decrease percentage NDFF in the grain. When applied with the second increment at this location, nitrapyrin decreased the amount of N in the whole plant. These observations suggest that the use of nitrification inhibitors cannot be considered risk-free insurance to reduce the potential for N losses from soils. They also suggest that increasing the potency or longevity of nitrification inhibitors may not be the best way to improve the benefits of using these inhibitors.

It is difficult to determine the exact nature of the observed adverse effects of nitrapyrin on plant growth. These adverse effects probably are associated with inhibiting nitrification and restricting the movement of fertilizer N in the soil. Inhibiting nitrification could induce a toxicity associated with high levels of ammonium or ammonia in the surface layer. Especially in soils having low concentrations of soil-derived N, restricting the movement of fertilizer N could discourage root growth below the surface layers and decrease the ability of the root system to forage for water and nutrients other than N. Because of the high potential for moisture stress that occurred in the summer of 1983, a decrease in the ability of roots to forage for water could explain the reductions in grain yields observed at Nashua. However, it should be emphasized that it is not possible to distinguish between nitrapyrin-induced toxicity effects and nitrapyrin-induced moisture stress in this study and, therefore, toxicity effects cannot be ruled out.

Data presented in Figure 2 provide additional evidence that nitrapyrin can have adverse effects on grain yields under some conditions. In this figure, yields are expressed relative to the highest yields observed within each site-year. This figure visually demonstrates that nitrapyrin had an adverse effect on yields because most of the points above the critical level and below the yield plateau are from the treatments that received nitrapyrin. Data presented in this figure also support the conclusion that an abundance of N must be considered a probable reason for the lack of increases in grain yields in response to addition of nitrapyrin in this study.

More than half of the responses (in Tables 4 through 7) that were statistically significant and also indicated that nitrapyrin increased the availability of fertilizer N occurred at Ames in 1982. This is somewhat disturbing because an integrated analysis of all data collected indicates that nitrapyrin had an adverse effect on plant growth at this site year. It is recognized that the soil at this site year was not representative of normal production practices because it had been cropped to continuous corn for several years without fertilization. However, the site at Nashua had a cropping history more typically found in production agriculture and the overall (years pooled) effect of nitrapyrin at this site was to significantly decrease yields.

Data presented by Touchton et al. (1979) indicate that nitrapyrin applied at DeKalb, Illinois in the spring of 1976 with 67 or 134 kg N ha<sup>-1</sup> as anhydrous ammonia decreased yields while the addition of more N increased yields. Like the soil at Nashua, the soil at DeKalb was

classified as poorly drained. Like the summer of 1983 at Nashua, moisture stress was apparently a significant factor affecting yields at DeKalb in 1976. Touchton et al. (1979) also reported no clear benefits of nitrapyrin applied with anhydrous ammonia at other sites located on poorly drained soils. Because of our results and those of Touchton et al. (1979), it is believed that greater attention should be given to distinguishing between anhydrous ammonia and other fertilizer materials as well as between well drained and poorly drained soils when evaluating the benefits of using nitrapyrin. I suggest that identifying situations in which nitrification inhibitors should not be used is a viable strategy for gaining acceptance of these compounds where they should be used for economic or environmental reasons.

## SUMMARY

The response of corn (Zea mays L.) to  $^{15}\text{N}$ -labeled anhydrous ammonia applied at 112 and 224 kg N ha $^{-1}$  with and without nitrapyrin was studied at two sites in Iowa during 1982, 1983, and 1984. Significant increases in grain yields were observed in response to N at both sites, but this response was only to the first increment at one site. Nitrapyrin had a statistically significant effect on grain yields at only two of the 12 rate-site years, and this effect was negative. An abundance of fertilizer-derived and soil-derived N must be considered a major reason for lack of yield responses to nitrapyrin. However, integrated analyses of the results of determinations of N content, percentage of N derived from fertilizer, and dry matter yields for various plant parts suggest that nitrapyrin sometimes had adverse effects on plant growth. The adverse effects may have been the result of nitrapyrin increasing the susceptibility of plants to moisture stress.

The results show that the use of nitrification inhibitors cannot be considered risk-free insurance to reduce the potential of N losses from soils. They suggest the best strategy for increasing the economic and environmental benefits of using nitrification inhibitors is to improve our ability to select the rates of N that are to be applied with these compounds and to avoid situations where nitrification inhibitors may have adverse effects.

PART IV. RECOVERY OF ANHYDROUS AMMONIA  $^{15}\text{N}$  DURING THREE YEARS OF  
CORN PRODUCTION



## INTRODUCTION

Numerous researchers have determined recovery of  $^{15}\text{N}$ -labeled fertilizers in soils and crops at one or more selected times after this N was applied to soils under field conditions. Such determinations are the only practical way to assess total amounts of fertilizer N lost to the environment under field conditions, where it is impossible to directly determine losses of N by leaching and denitrification. Knowledge of the amounts of fertilizer N lost during crop production is important for both environmental and agronomic reasons.

Although the use of  $^{15}\text{N}$ -labeled fertilizers enables unequivocal determinations of N recovery in crops and soils at any given time after fertilization, several factors related to methodology have a major impact on determined values for percentage recovery and on the context in which these values are significant. One of these factors relates to selection of the time at which recovery is determined. Many researchers (Carter et al., 1967; Westerman et al., 1972; Bigeriego et al., 1979; Olson, 1980b) studying recovery of labeled fertilizers during production of corn (Zea mays L.) or sudangrass (Sorghum sudanense L.) have determined recovery in the crop and soil at the time of harvest. This practice shows the amounts of N lost during the growing season and, therefore, is a direct way to evaluate the efficiency of a fertilization practice for the first crop after fertilization. However, this method does not provide information concerning the amounts of fertilizer N lost between cropping seasons or the residual value of this fertilizer N to

future crops. Therefore, it cannot be used to evaluate the overall or long-term efficiency of a fertilization practice or the total amounts of fertilizer N lost to the environment.

The residual value of fertilizer N during crop production can be directly determined by using  $^{15}\text{N}$ -labeled fertilizers. However, the determined value of residual N should be expected to vary with the amounts of N applied after the labeled fertilizer was applied. Some researchers (Westerman and Kurtz, 1972; Chichester and Smith, 1978;) have assessed the residual value of N fertilizers by measuring recovery of fertilizer N in two or more sequential crops without applying more fertilizer. This practice is satisfactory for studying the rate at which fertilizer N becomes available following transformations that make it unavailable in the soil. However, the residual values observed in such studies may not be applicable to the residual value of these fertilizers in production agriculture, where fertilizer is applied to meet crop needs each year. Similarly, the practice of covering plots with plastic to prevent losses by leaching or denitrification between cropping seasons (Carter et al., 1967; Westerman and Kurtz, 1972) is a valid way to determine the potential for carry over of fertilizer N from one crop to the next. However, the residual values obtained by this method may not be applicable to production agriculture where these losses normally occur.

Despite the large amounts of fertilizer N used for corn production in the Corn Belt and the large potential for N losses between cropping seasons in this region, I can find no published reports of field studies

conducted in this region to determine recovery of  $^{15}\text{N}$ -labeled fertilizer in soils and corn tissue over periods of one or more years in which N fertilizers are applied at recommended rates. Such studies are needed to assess the potential threats of fertilizer N to the environment and to evaluate the overall or long-term efficiency of current N fertilization practices.

Reported here are studies that directly address this need. I used isotope tracers to determine recovery of N applied as anhydrous ammonia with and without nitrapyrin over periods of one, two, and three years. Anhydrous ammonia is the most widely used N fertilizer in the Corn Belt and nitrapyrin is a nitrification inhibitor often applied with anhydrous ammonia to reduce losses of N by leaching and denitrification (Keeney, 1980; Meisinger et al., 1980; Hauck, 1983).

## MATERIALS AND METHODS

The studies were conducted at the Northeast Iowa Research Center near Nashua and at the Agronomy and Agricultural Engineering Research Center near Ames. The design of the experiment is described in a previous paper (see Part III). Briefly, the study includes two N rates (112 and 224 kg N ha<sup>-1</sup> as anhydrous ammonia) with and without nitrapyrin applied to the same plots for each of three years. Two types of plots were used, yield plots and <sup>15</sup>N plots within the yield plots. The <sup>15</sup>N plots received the same treatments as the yield plots except that isotopically labeled anhydrous ammonia was substituted for unlabeled ammonia. The <sup>15</sup>N plots were established at different locations within the yield plots in 1982, 1983, and 1984.

Soil samples were collected from all plots (<sup>15</sup>N and yield plots) to a depth of 150 cm (individual samples representing 0-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, and 120-150 cm) before fertilizer treatments were applied in the springs of 1982, 1983, and 1984. Similar samples were collected from all plots in the spring of 1985. Each sample was a composite of 8 cores. These samples were air-dried before storage for subsequent analyses.

Exchangeable ammonium-N and (nitrate plus nitrite)-N contents of each soil sample were determined by extraction with 2 N KCl and steam distillation with magnesium oxide and Devarda alloy as described by Keeney and Nelson (1982). Because distillates from these analyses were used for <sup>15</sup>N determinations, 5 ml of an ammonium nitrate standard

containing 15 ug ammonium-N ml<sup>-1</sup> and 15 ug nitrate-N ml<sup>-1</sup> was added to each aliquot (20 ml) of soil extract distilled. This practice assured that each sample contained enough N to be within the working range of the mass spectrometer used for <sup>15</sup>N determinations. To avoid cross-contamination of samples by ammonium exchange processes on the condenser (see Hauck, 1982), separate distillation systems were used for ammonium-N and (nitrate plus nitrite)-N distillations and duplicate aliquots of each soil extract were analyzed. Distillates from the first aliquots were collected in boric acid indicator solution and then titrated with acid as described by Keeney and Nelson (1982). Distillates from the second aliquots were collected in 2 ml of 0.08 N H<sub>2</sub>SO<sub>4</sub>, concentrated (by evaporation of water) to a volume of about 2 ml, and stored in 2-dram vials.

Plant samples were collected from <sup>15</sup>N plots and yield plots at physiological maturity and separated into grain and stover components. Except for small amounts used for analyses, the stover was returned to the plots. A special effort was made to return the <sup>15</sup>N-labeled stover to the <sup>15</sup>N plots. To assure that these residues remained in place, the <sup>15</sup>N plots were covered with poultry wire until the fields were plowed. Plant materials to be used for analyses were dried at 65°C and ground for analysis. The permanganate-reduced iron modification of the Kjeldahl procedure (Bremner and Mulvaney, 1982) was used to determine N contents of soil and tissue samples.

Determinations of <sup>15</sup>N in soils, soil extracts, and plant residues were performed by reacting the concentrated distillates with sodium

hypobromite in evacuated Rittenberg flasks as described by Hauck (1982) and injecting the resulting dinitrogen gas into a Varian MAT 250 mass spectrometer. Atom percentages  $^{15}\text{N}$  in these distillates (A) were calculated by equation 1,

$$A = 100/[2(I_{28}/I_{29}) + 1] \quad (1)$$

where  $I_{28}$  and  $I_{29}$  represent ion currents at m/e 28 and 29, respectively.

Concentrations ( $\text{mg N kg}^{-1}$  soil) of fertilizer-derived nitrate-N ( $C_{fn}$ ) were calculated by equation 2,

$$C_{fn} = C_n(A_{en} - A_{rn})/(A_f - A_{rn}) \quad (2)$$

where  $C_n$  represents the concentration ( $\text{mg N kg}^{-1}$  soil) of nitrate-N in the soil, and  $A_f$ ,  $A_{en}$  and  $A_{rn}$  represent the atom percentages  $^{15}\text{N}$  of the fertilizer applied, nitrate extracted from the soil, and of distillates from the reference nitrate (75 ug of the standard nitrate-N in KCl), respectively. Atom percentages  $^{15}\text{N}$  in nitrate extracted from soils were calculated by equation 3,

$$A_{en} = (Q_{sn}A_{sn} - 75A_{rn}) / (Q_{sn} - 75) \quad (3)$$

where  $Q_{sn}$  represents quantity (ug) of nitrate-N in samples (soil extracts plus 75-ug of the standard-N), and  $A_{sn}$  represents atom percentages  $^{15}\text{N}$  in distillates of these samples. Concentrations of fertilizer-derived ammonium-N were obtained by similar calculations.

Concentrations of fertilizer-derived total-N ( $C_{ft}$ ) were calculated by equation 4,

$$C_{ft} = C_t (A_a - A_b) / (A_f - A_b) \quad (4)$$

where  $C_t$  represents concentration of total N found in samples collected at the end of the study and  $A_a$  and  $A_b$  represent atom percentages  $^{15}\text{N}$  of

soil N collected after and before labeled fertilizer was applied, respectively.

Statistical calculations followed Snedecor and Cochran (1967) and Steel and Torrie (1960).

## RESULTS AND DISCUSSION

Table 1 shows a summary of the effects of N rate and nitrapyrin on percentage recovery of labeled N in corn grain harvested during 1982, 1983, and 1984. The amount of N recovered in the grain is of special significance because, as is frequently done during corn production, corn grain was the only plant material removed from the plots in this study. Two observations are clear from the data presented in Table 1. First, only 13 to 33% (mean of 20%) of the labeled N was removed from the plots during corn harvest during the first crop following fertilization. Higher recoveries might have been observed if drought stress had not limited crop yields each year (see Part III). However, these values for percentage recovery were similar to values found by other workers (Chichester and Smith, 1978; Olson, 1980; Kitur et al., 1984; and Meisenger et al., 1985), who also used  $^{15}\text{N}$ -labeled fertilizers to determine recovery of fertilizer N in corn grain. Second, only small percentages (0.3 to 1.5%) of the labeled N were recovered in the second and third crops after application. These findings indicate that a substantial portion (65 to 85%) of the fertilizer N was not recovered during grain harvest.

Table 2 shows the effects of N rate and nitrapyrin on amounts of labeled N found in the grain and whole plant in the first, second, and third crops after fertilization. This table is arranged to enable presentation of statistical data concerning these effects within locations and number of crops after fertilization. This presentation is



Table 1. Percentage of fertilizer N recovered in corn grain as affected by N rate and nitrification inhibitor

Date applied	Location	N rate	1982		1983		1984	
			-NI	+NI	-NI	+NI	-NI	+NI
		kg ha <sup>-1</sup>	-----% of N applied-----					
1982	Ames	112	16	32	0.8	1.0	0.5	0.5
		224	19	19	0.6	0.7	0.4	0.4
	Nashua	112	20	22	0.6	0.7	0.6	0.6
		224	15	13	0.6	0.3	0.4	0.4
1983	Ames	112	na	na	20	17	0.9	1.0
		224	na	na	15	20	0.8	0.9
	Nashua	112	na	na	16	14	1.4	1.5
		224	na	na	14	13	1.1	1.0
1984	Ames	112	na	na	na	na	21	23
		224	na	na	na	na	17	20
	Nashua	112	na	na	na	na	33	30
		224	na	na	na	na	18	19

Table 2. Recovery of fertilizer N in corn plants as affected by N rate and nitrification inhibitor

N Rate	NI	Ames		Nashua	
		In grain	Whole plant	In grain	Whole plant
kg N ha <sup>-1</sup>		-----kg N ha <sup>-1</sup> -----			
<u>First crop following fertilization<sup>a</sup></u>					
112	-	21.6	38.7	25.8	48.2
112	+	27.2	49.5	24.8	49.7
224	-	37.9	64.7	35.4	71.8
224	+	44.4	75.4	33.9	66.7
Statistical data:					
N Rate		0.01 <sup>b</sup>	0.01	0.01	0.01
NI		0.01	0.01	NS	NS
N Rate * NI		NS	NS	NS	NS
CV (%)		20	18	20	15
<u>Second crop following fertilization<sup>c</sup></u>					
112	-	1.0	1.6	1.2	1.9
112	+	1.1	1.9	1.2	2.1
224	-	1.6	2.9	1.9	3.5
224	+	1.9	3.2	1.6	3.2
Statistical data:					
N Rate		0.01 <sup>b</sup>	0.01	0.01	0.01
NI		NS	NS	NS	NS
N Rate * NI		NS	NS	NS	NS
CV (%)		31	29	31	28

<sup>a</sup>Values are means averaged over 1982, 1983, and 1984.

<sup>b</sup>NS indicates a probability level >0.10.

<sup>c</sup>Values are means averaged over 1983 and 1984.

Table 2. Continued

N Rate	NI	Ames		Nashua	
		In grain	Whole plant	In grain	Whole plant
kg N ha <sup>-1</sup>		-----kg N ha <sup>-1</sup> -----			
<u>Third crop following fertilization<sup>d</sup></u>					
112	-	0.5	0.7	0.7	1.1
112	+	0.7	1.0	0.7	1.1
224	-	0.8	1.3	0.8	1.5
224	+	0.9	1.4	0.9	1.7
Statistical data:					
N Rate		0.01	0.01	0.05	0.01
NI		0.03	0.10	NS	NS
N Rate * NI		NS	NS	NS	NS
CV (%)		16	18	14	18

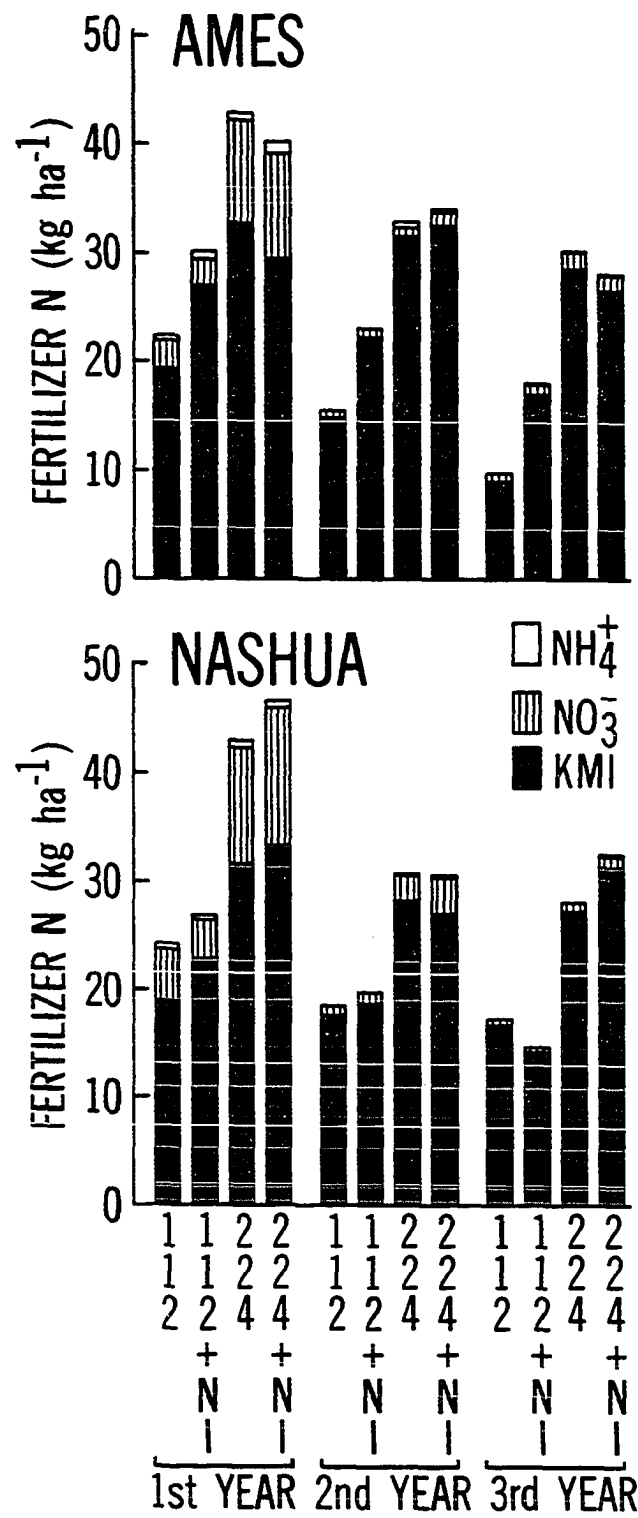
<sup>d</sup>Values are means for 1984.

valid because I found no significant year by treatment (N or nitrification inhibitor) interactions.

Increasing the rate of application of labeled anhydrous ammonia from 112 to 224 kg N ha<sup>-1</sup> increased uptake of labeled N in both the grain and whole plant during the first, second, and third crops after fertilization at both locations. Nitrapyrin increased uptake of labeled N in grain and whole plants at the Ames location in the first crop. This effect was greatest in 1982, the growing season in which soil moisture was least limiting (see Part III). Nitrapyrin had no significant effect on the uptake of labeled N at the Nashua location. The data presented show that nitrapyrin had negligible effects on the amounts of labeled N recovered by plants in the second and third growing season. This observation does not support the suggestion of Ashworth (1986) that nitrification inhibitors should be regarded as tools for decreasing next season's N requirement rather than tools for increasing this year's yield.

The amounts of labeled N found at various times in the surface 1.5 m of soil as nitrate, exchangeable ammonium, and as the fraction we refer to as KMI-N (Kjeldahl minus inorganic) are shown in Figure 1. The term "KMI-N fraction" is used because it includes N from both organic matter and nonexchangeable ammonium and because no attempt was made to distinguish between these forms. The organic matter could be soil organic matter, microbial biomass, or plant residues. The total amount of labeled N found in the soil one year after fertilization accounted for 19 to 23% of that applied. Increasing the rate of N application

Figure 1. The amounts of labeled N in the surface 1.5 m of soil found at various times after this N was applied



significantly ( $P > 0.01$  for the first and second years,  $P > 0.10$  for the third year) increased the total amount of labeled N found in the soil one, two, and three years after fertilization at both locations. This increase was largely due to the amounts of labeled N in the KMI-N fraction, which accounted for 71 to 89% of the total amounts of labeled N recovered in the soils. This observation is consistent with the conclusions of Allen et al. (1973), Olson (1980), and Power and Legg (1984), who concluded that most of the fertilizer N that remains in the soil after the first cropping season is in the organic form.

Only small portions of the labeled N were found in the soil as mineral N, with nitrate and exchangeable ammonium accounting for 12 to 27% and 2 to 3% of the labeled N found in the soil after the first year, respectively. Rate of N application had statistically significant effects ( $P > 0.05$ ) on the amounts of labeled N found as nitrate the first, second, and third years after fertilization, but these increases represented trivial amounts of N in the second and third years. Rate of N application had no significant effects on amounts of labeled N found as exchangeable ammonium. Nitrapyrin had no significant effect on amounts of labeled N recovered as nitrate or exchangeable ammonium. Data presented in Tables 3 and 4 show that neither N rate nor nitrapyrin had marked effects on the distribution of labeled ammonium or nitrate in the soil. These observations suggest that either little fertilizer-derived nitrate N remained in the soil at harvest of each crop or that most of the nitrate N remaining at harvest was lost by leaching or denitrification during the fall-to-spring period.

Table 3. Concentrations of labeled ammonium-N found at various depths one, two, and three years after fertilization as affected by N rate and nitrification inhibitor

[illegible]



Table 4. Concentrations of labeled nitrate found at various depths one, two, and three years after fertilization as affected by N rate and nitrapyrin

Depth	Ames				Nashua			
	112-NI	112+NI	224-NI	224+NI	112-NI	112+NI	224-NI	224+NI
--cm--	-----mg N kg soil <sup>-1</sup> -----							
	<u>First Year</u>							
0-12	0.1	0.1	0.2	0.3	0.2	0.1	0.2	0.3
12-24	0.1	0.1	0.2	0.2	0.2	0.2	0.3	0.4
24-36	0.1	0.1	0.1	0.2	0.2	0.2	0.4	0.6
36-48	0.1	0.1	0.1	0.3	0.2	0.2	0.7	0.6
48-72	0.1	0.2	0.2	0.3	0.2	0.2	0.6	0.8
72-96	0.2	0.2	0.5	0.7	0.2	0.2	0.6	0.8
96-120	0.1	0.1	0.7	0.6	0.2	0.2	0.5	0.6
120-150	0.1	0.1	0.6	0.4	0.3	0.2	0.3	0.5
	<u>Second Year</u>							
0-12	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
12-24	<0.1	<0.1	0.1	0.1	0.1	0.1	0.1	0.2
24-36	<0.1	<0.1	0.1	0.1	0.1	0.1	0.1	0.1
36-48	<0.1	0.1	<0.1	0.1	0.1	0.1	0.1	0.1
48-72	<0.1	<0.1	<0.1	0.1	<0.1	0.1	0.1	0.1
72-96	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.1	0.2
96-120	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.1	0.2
120-150	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.1	0.2
	<u>Third Year</u>							
0-12	<0.1	0.1	0.1	0.1	<0.1	<0.1	0.1	0.1
12-24	<0.1	<0.1	0.1	0.1	<0.1	0.1	0.1	0.1
24-36	<0.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1	0.1
36-48	<0.1	0.1	0.1	0.1	<0.1	<0.1	0.1	<0.1
48-72	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	0.1	<0.1
72-96	<0.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
96-120	<0.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1	<0.1
120-150	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1

Although the amounts of labeled N in the KMI-N fraction increased with rate of fertilization, the percentage recovery of labeled N decreased with rate of fertilization. This relationship suggests that increasing the concentration of fertilizer may have promoted losses of N by ammonia volatilization, saturated sites in clay materials that fix ammonium, or inhibited microorganisms that immobilize N into organic fractions. This observation is consistent with the conclusion of Olson (1980), who reported that the soil's capacity to immobilize mineral N is limited. Because large portions (47 to 94%) of the labeled N that were found in the KMI-N fraction one year after fertilization were still present three years after fertilization, it must be concluded that release of labeled N from the KMI-N fraction is quite slow after the first cropping season. This conclusion is similar to that of Allen et al. (1973) and is consistent with our observation that small amounts of labeled N were recovered by the second and third crops after fertilization. It also explains the presence of small amounts of labeled mineral N into the third cropping season.

When nitrapyrin was applied with  $112 \text{ kg N ha}^{-1}$  at Ames, this compound significantly ( $P > 0.01$ ) increased the amounts of labeled N found in the KMI-N fraction (Figure 1). This observation is consistent with the conclusions of Juma and Paul, (1983) who suggested that nitrification inhibitors may increase net immobilization by delaying nitrification. However, nitrapyrin had no significant effect on the amounts of labeled N found in the KMI-N fraction at this location when  $224 \text{ kg N ha}^{-1}$  was applied or at the Nashua location when either rate of

N was applied. The failure of nitrapyrin to increase the amount of labeled N that moved into the KMI-N fraction under some conditions could be explained if it is assumed that soils have a limited capacity to fix or immobilize N. Therefore, the ability of nitrapyrin to conserve fertilizer N by inducing immobilization or fixation may not be important when this compound is applied to soils having high levels of available N or when it is applied with high concentrations of fertilizer.

Figure 2 shows the total amounts of nitrate and exchangeable ammonium found in the surface 1.5 m of soil immediately before fertilizers were applied each year. The N shown in this figure could be derived from soil, labeled fertilizers, or unlabeled fertilizers. Nitrapyrin had no significant effects on amounts of nitrate or exchangeable ammonium present. This observation indicates that our inability to see effects nitrapyrin on the amounts of  $^{15}\text{N}$  in the mineral form cannot be attributed to errors caused by isotope exchange reactions in the soil.

Observed differences in amounts of mineral N are largely caused by variations in amounts of nitrate, since there were no significant differences in amounts of exchangeable ammonium among years or between N rates within years. At the Ames location, there were significant ( $P>0.09$ ) differences in amounts of nitrate found among years. However, there were no consistent changes with time. In the springs of 1984 and 1985, there was significantly ( $P>0.04$ ) more nitrate in plots having the higher rate of N. At the Nashua location, there also were significant ( $P>0.01$ ) differences in amounts of nitrate found among years. However,

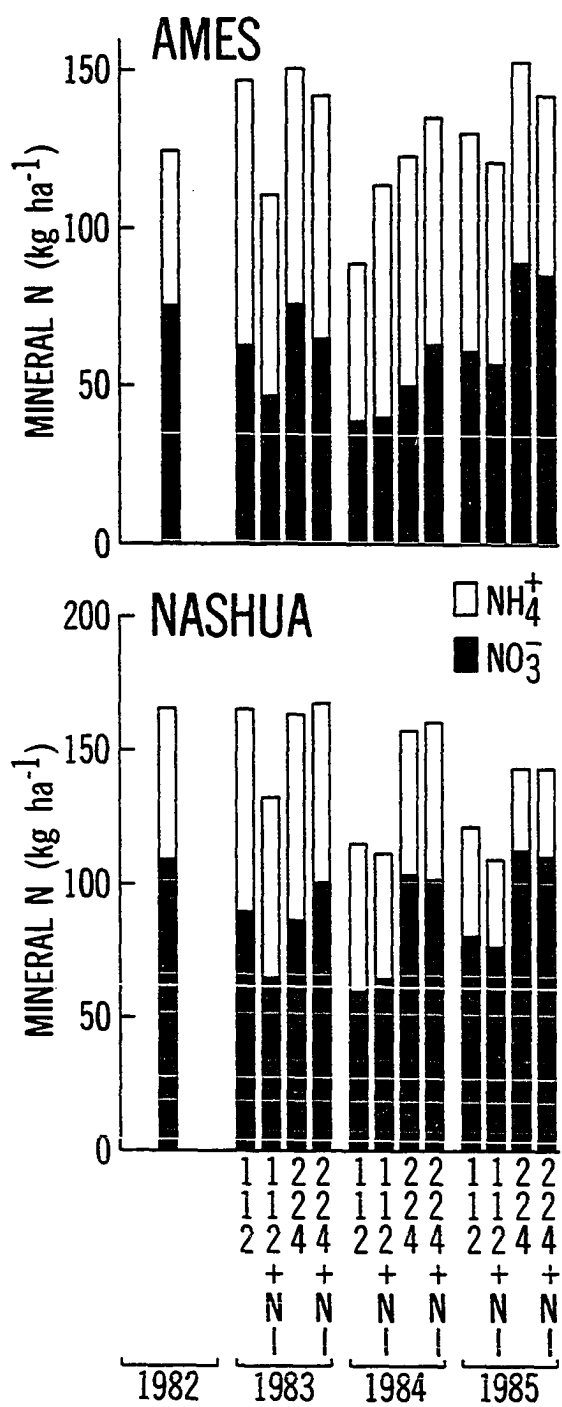


Figure 2. The amounts of mineral N found in the surface 1.5 m of soil immediately before fertilizers were applied each spring (NI= nitrification inhibitor)

especially at the lower rate of fertilization, there was a gradual decrease in amount with time. In the springs of 1984 and 1985, there was significantly ( $P>0.01$ ) more nitrate in plots having the higher rate of N. These observations suggest that sequential additions of as much as  $224 \text{ kg N ha}^{-1}$  did not result in a progressive increase in amounts of mineral N. Figure 3 shows that these additions of fertilizer N did not result in a progressive change in the distribution of mineral N in the surface 1.5 m of soil. These observations agree with those of Nelson and MacGregor (1973) and Overdahl et al. (1980), who studied the effect of N fertilization on nitrate distributions in similar soils in Minnesota and concluded that little accumulation of nitrate was observed in the surface layer of soil when fertilizers were applied at normal rates.

Data presented in Table 5 show that a substantial portion (49 to 64%) of the labeled N could not be accounted for one year after fertilization. Because low recoveries of fertilizer N could not be attributed to errors caused by lateral movement of labeled N (see Part II), it must be concluded that the unrecovered N was lost from the surface 1.5 m layer of soil. Intensive studies (see Part V) of the distribution of labeled N during the growing season showed evidence that rapid downward movement of nitrate occurred. Therefore, leaching as well as denitrification must be considered probable mechanisms of N loss.

I believe that a large portion of the fertilizer N was lost from the soil during the fall-to-spring period. Losses of labeled N by

Figure 3. Distributions of mineral N found immediately before fertilizers were applied each spring. Data are shown only for the plots receiving 224 kg N ha<sup>-1</sup> each year

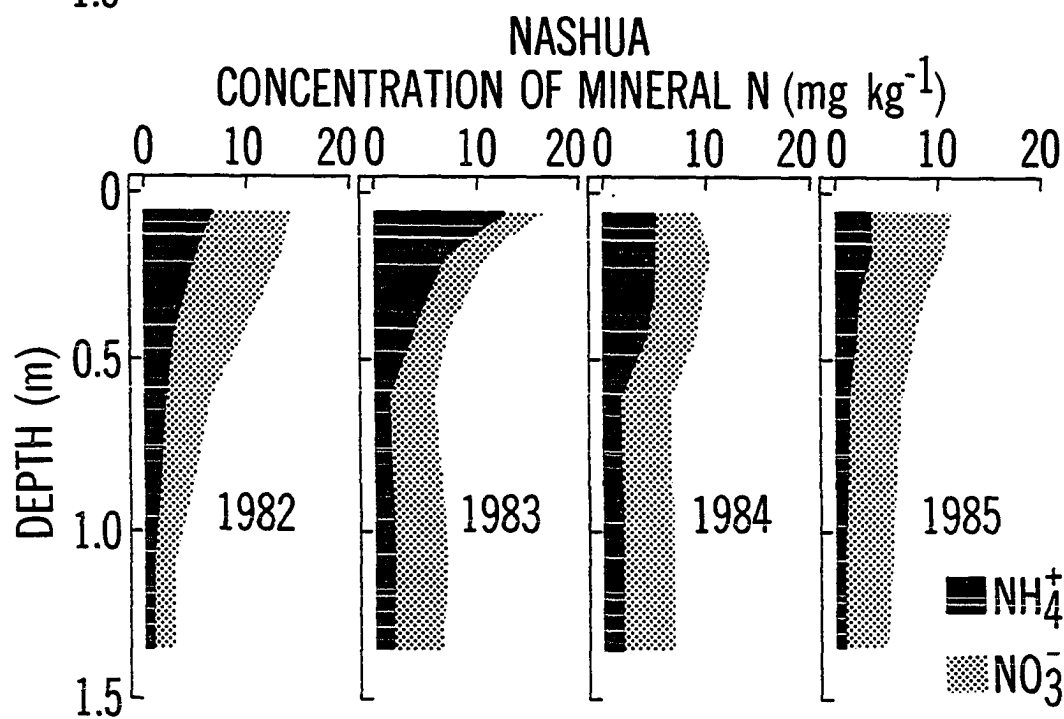
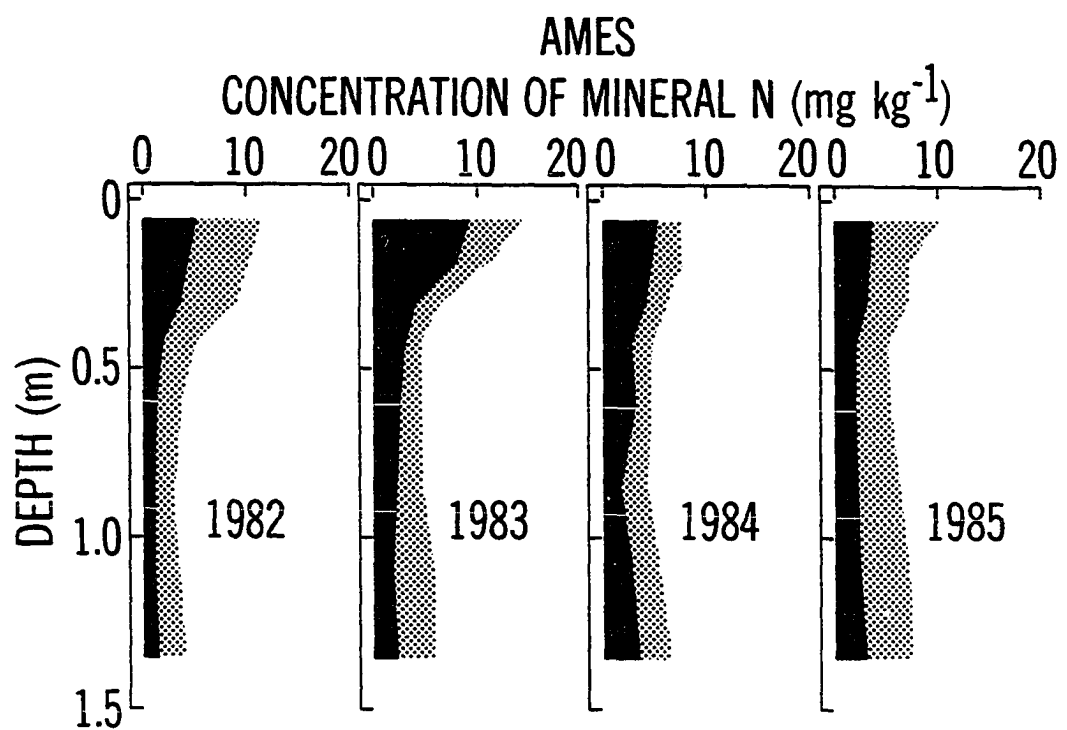


Table 5. Percentage of fertilizer N accounted for one year after fertilization as affected by N rate and nitrification inhibitor

N rate	NI	Ames				Nashua			
		1982	1983	1984	Mean	1982	1983	1984	Mean
------%-----									
112	-	35	46	38	39	36	38	62	45
112	+	54	46	54	51	38	39	61	46
224	-	37	34	37	36	27	37	45	35
224	+	36	39	38	38	29	37	41	36



leaching and (or) denitrification during the fall-to-spring period could explain why the recoveries reported here are lower than those observed by other workers (Carter et al., 1967; Westerman et al., 1972; Bigeriego et al., 1979; Olson, 1980), who assessed recovery of labeled N by determining amounts of labeled N in the soil immediately after crop harvest. In field studies, several workers (Cameron et al., 1978; Bauder and Montgomery, 1979; Malhi and Nyborg, 1983) have found N losses to be substantial between late fall and early spring period and I believe a realistic assessment of fertilizer recovery must include the amounts of N lost during this period.

Another possible reason why the recoveries of labeled N observed in this study were lower than those observed by other workers is that, unlike previous workers, I assessed recovery when N was applied at the same rate each year. Certainly, my recoveries during the second and third crops would be expected to be higher if I had not applied additional (unlabeled) N for these crops. Since fertilizers are usually applied at similar rates each year, I believe that the most realistic assessments of fertilizer recovery are obtained when fertilizer N is applied at continuous rates.

## SUMMARY

Studies were conducted at two locations to determine recovery of  $^{15}\text{N}$ -labeled anhydrous ammonia with and without nitrapyrin during corn production over a three-year period in which N fertilizers were applied annually at rates commonly used in the Corn Belt. The results showed that 13 to 33% of the labeled N was removed from the plots during corn harvest in the first crop after fertilization. Although the total amounts of labeled N found in the whole plants ranged from 29 to 45%, the stover was returned to the plots. Nitrapyrin increased the amount of N recovered in plant tissue at only one of the six site-years studied.

Only small percentages (0.3 to 1.5%) of the labeled N were recovered in the second and third crops after fertilization. Nitrapyrin had negligible effects on the amounts of labeled N recovered by plants in the second and third growing seasons. These findings suggest that fertilizer N applied for one cropping season has little residual value in subsequent cropping seasons where fertilizers are applied each year.

Analyses of soil samples collected one year after fertilization showed that about 19 to 23% of the fertilizer N remained in the soil. Only small portions of this N were as exchangeable ammonium and nitrate. Most of this N was in the KMI-N (kjeldahl minus inorganic) fraction, which includes N from both organic matter and nonexchangeable ammonium. Large portions (47 to 94%) of the labeled N that were found in the KMI-N fraction one year after fertilization were still present three years

after fertilization.

The results of this study showed that a substantial portion (49 to 64%) of the labeled N was lost from the surface 1.5 m of soil during the first year by processes other than plant uptake. These losses of fertilizer N are greater than have been reported from many comparable studies. A probable explanation for this difference is that my determinations included the amounts of fertilizer N lost during the fall-to-spring period. These losses must be considered when evaluating the long-term efficiency of N fertilization practices and the effects of these practices on environmental quality.

PART V. DISTRIBUTION AND RECOVERY OF ANHYDROUS AMMONIA

<sup>15</sup>N IN THE ROOTING ZONE OF CORN

## INTRODUCTION

Anhydrous ammonia that is injected into soil immediately reacts with the soil solution to form ammonium and hydroxyl ions, a reaction that increases soil pH. The ammonium ions are attracted to cation exchange sites on soil particles and are retained near the point of ammonia injection. There are several reports (Blue and Eno, 1954; McIntosh and Frederick, 1958; Cochran et al., 1973; Chalk et al., 1975; Hendrickson et al., 1978a;b; Touchton et al., 1978; Hogg and Henry, 1982) describing distributions of ammonium and pH values within the ammonia retention zone at various times following application of anhydrous ammonia to soils under field conditions. The ammonia retention zone is usually considered to be within 10 cm of the point of ammonia injection.

Determinations of ammonium, nitrite, and (or) nitrate concentrations within ammonia retention zones at various times after fertilization have often been used to assess rates of nitrification of fertilizer N in soils and (or) to evaluate the effectiveness of various nitrification inhibitors under field conditions (Hughes and Welch, 1970; Touchton et al., 1978; Hendrickson et al., 1978). This method of assessing rates of nitrification may have significant errors because ammonium concentrations may decrease as a result of several processes, including ammonia volatilization (Du Plessis and Kroontje, 1964), ammonia fixation by soil organic matter (Mortland, 1958; Burge and Broadbent, 1961; Nommik and Nilsson, 1963b; Broadbent and Stevenson,

1966; Stevenson, 1982), fixation of ammonium into clay minerals (Nommik, 1957; Mortland, 1966; Kowalenko and Cameron, 1976), or immobilization into organic matter by microbial activities (Frederick and Broadbent, 1966; Legg et al., 1971; Jansson and Persson, 1982). Although there are often large discrepancies between amounts of ammonia injected into soils and amounts of ammonium found shortly after this injection, there is little evidence to indicate which of these processes is most responsible for the discrepancies.

Nitrate, the major product of nitrification, is relatively mobile in soils and can be lost from soils by denitrification or by leaching. Because nitrate can move by diffusion or convection in soils and because the ammonia retention zone represents only a small fraction of the rooting zones of many crops, measurements of nitrate losses from the ammonia retention zone cannot be used to assess losses of N by leaching or by denitrification under many field conditions.

Reported here are the results of  $^{15}\text{N}$ -tracer studies of the distribution and recovery of anhydrous ammonia-derived N within the rooting zone of corn. The rationale for this work was that use of  $^{15}\text{N}$ -labeled ammonia would enable studies of the incorporation of fertilizer N into organic and mineral forms within soils as well as assessments of the movements of fertilizer-derived N outside the ammonia retention zone. The effects of nitrapyrin were evaluated because this compound is often applied with anhydrous ammonia to prevent losses of N by leaching and denitrification.

## MATERIALS AND METHODS

Small plots (2 by 2 m) were established in duplicate on Clarion (fine-loamy, mixed, mesic, Typic Hapludolls) and Webster (fine-loamy, mixed, mesic Typic Haplaquolls) soils at the Agronomy and Agricultural Engineering Research Center near Ames, Iowa (see Table 1). The Webster soil is classified as being poorly drained while the Clarion soil is classified as being well drained. On 20 May, 1983, the plots were fertilized with  $^{15}\text{N}$ -labeled anhydrous ammonia and  $^{15}\text{N}$ -labeled anhydrous ammonia plus nitrapyrin with the method described in Part I. The anhydrous ammonia had 4.5 atom percent  $^{15}\text{N}$  and was applied at a rate equivalent of  $224 \text{ kg N ha}^{-1}$ . The plots were planted to corn. The bands were positioned in such a way that each band was halfway between two rows of corn that were 75 cm apart.

After 45 and 90 days, portions of each plot were excavated to obtain soil samples that were located at various positions relative to the fertilizer bands. The portion excavated was 75 cm wide (between two corn rows), 15 cm long (along the fertilizer bands), and 37.5 cm deep. The soil was carefully removed using spatulas and small digging tools to yield 50 separate soil samples from various locations as illustrated in Figure 1. An auger was used to take another 20 samples of soil at depths between 37.5 and 150 cm as shown in Figure 1. Each sample was individually air-dried, sieved, and thoroughly mixed. Portions of each sample were placed in plastic-lined bags and stored for analyses.

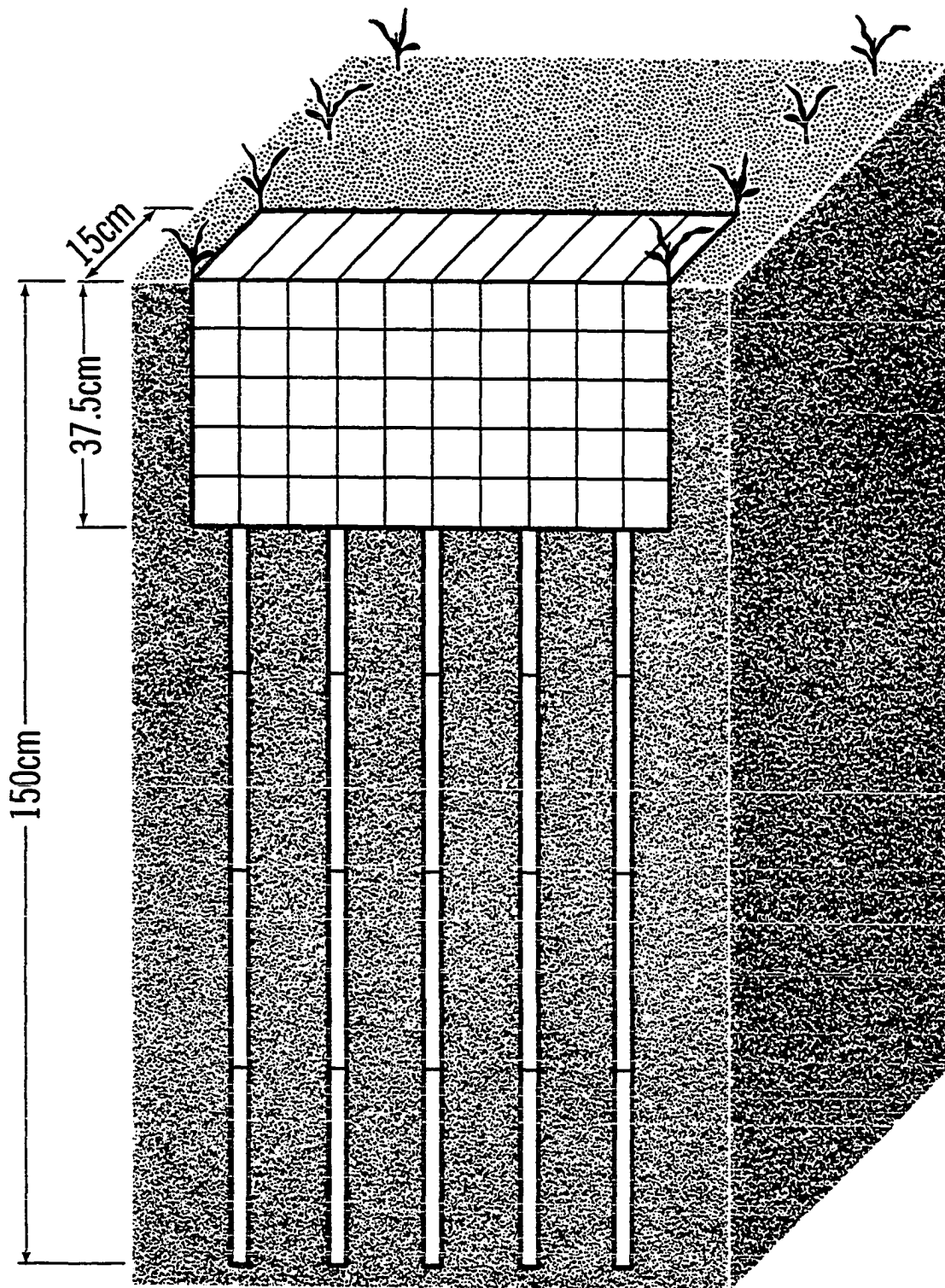
Exchangeable ammonium-N and (nitrate plus nitrite)-N contents of

Table 1. Selected properties of soils used in study

	pH	CEC	Sand	Silt	Clay	Total C	Total N
		cmol kg <sup>-1</sup>	-----	%-----	-----	g kg <sup>-1</sup> -----	
Webster	5.7	31	34	36	30	29	2.7
Clarion	6.8	17	51	31	18	22	1.4



Figure 1. Diagram showing location of samples collected from the soil profile



each soil sample were determined by extraction with 2 N KCl and steam distillation with magnesium oxide and Devarda alloy as described by Keeney and Nelson (1982). Because distillates from these analyses were used for  $^{15}\text{N}$  determinations we used a double distillation process described in Part IV. Plant materials to be used for analyses were dried at  $65^{\circ}\text{C}$  and ground for analysis. The permanganate-reduced iron modification of the Kjeldahl procedure (Bremner and Mulvaney, 1982) was used to determine N content of soil and tissue samples. The resulting distillates were prepared for isotope ratio analysis by using the method of Hauck (1982). Determinations of isotope ratios in soils, soil extracts, and plant residues were performed by using a Finnigan MAT 250 mass spectrometer. The amounts of labeled fertilizer in each sample were calculated as described in Part IV. Determinations of pH in soil were performed using 0.01 N  $\text{CaCl}_2$  as described by McLean (1982).

Isoconcentration lines for fertilizer-derived ammonium N, nitrate N, KMI-N (Kjeldahl minus inorganic), and soil pH were obtained by using the CONTR 2 program that is available at the Iowa State University Computation Center. Before isoconcentration lines were plotted for the fertilizer-derived nitrate, the data were interpolated using a IMSL subroutine called IQHSCV to give a uniform sampling grid to a depth of 150 cm.

## RESULTS AND DISCUSSION

The amounts of rainfall that occurred at the site during May, June, July, and August 1983 were 157, 232, 98, and 107 mm, respectively. The corresponding long-term means are 109, 132, 84, and 98 mm. Therefore, the samples collected at 45 days were collected after a period that was above average in amount of rainfall, and the samples collected at 90 days were collected after a period that was about average in amount of rainfall.

The concentration distribution patterns of fertilizer-derived N in various fractions are shown in Figures 2 through 5. Data are shown only for selected soil profiles. Because of the nature of the data, it is more meaningful to provide selected figures rather than figures that represented means across replications. The figures were selected to represent the range of distributions observed, rather than the similarity of replications. The isoconcentration lines shown in each figure were selected to give the clearest individual figures, rather than uniformity among figures.

Figures 2 and 3 show selected concentration distribution patterns for fertilizer-derived exchangeable ammonium found in Webster and Clarion soils, respectively. The isoconcentration lines show that fertilizer N as ammonium moved little from the point of injection. The isoconcentration lines form a series of concentric irregular circles, with the highest concentrations near the point of injection. These observations are consistent with earlier descriptions of ammonium

Figure 2. Concentration ( $\text{mg N Kg}^{-1}$  soil) distributions for fertilizer-derived ammonium in the Webster soil

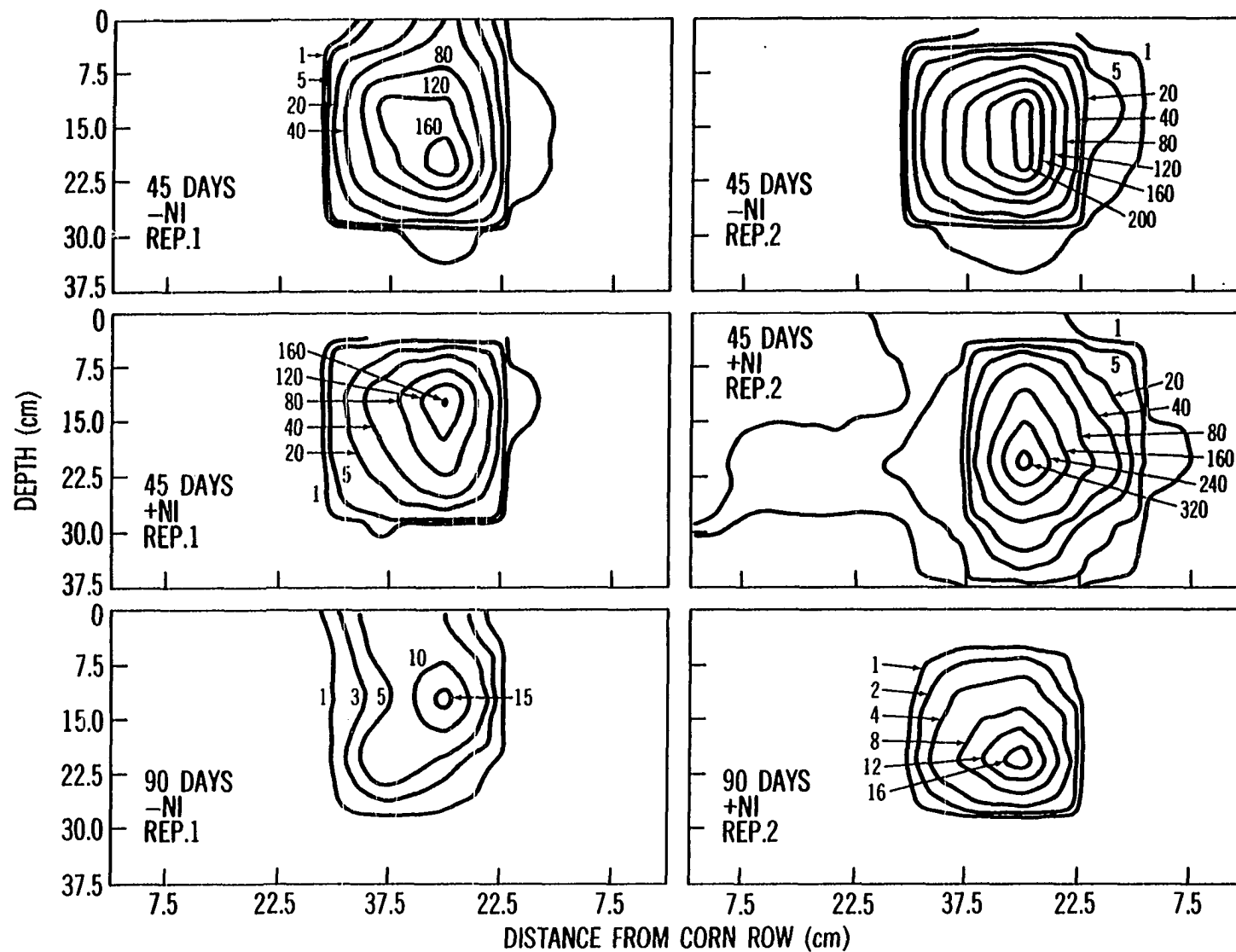


Figure 3. Concentration ( $\text{mg N Kg}^{-1}$  soil) distributions for fertilizer-derived ammonium in the Clarion soil

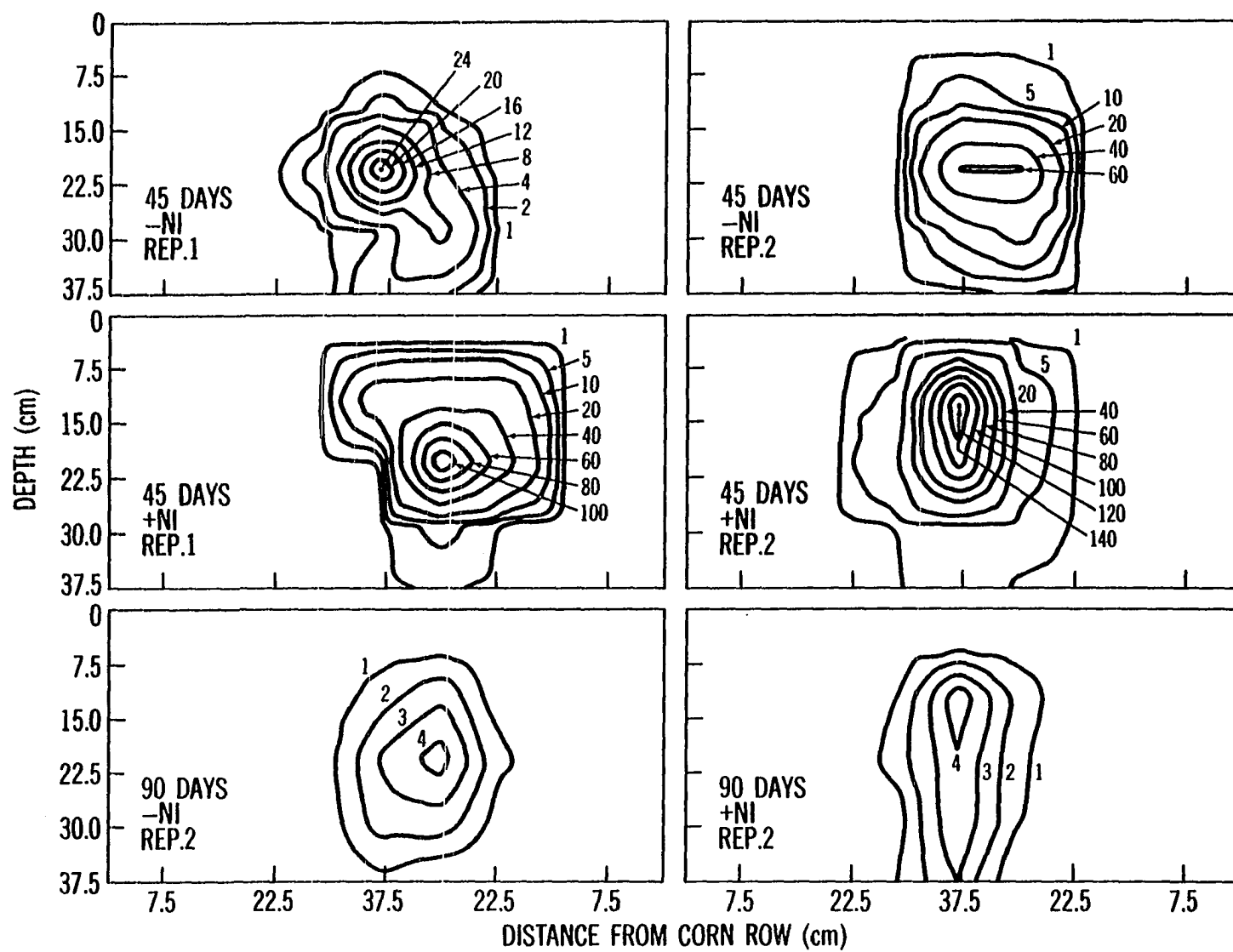




Figure 4. Concentration ( $\text{mg N Kg}^{-1}$  soil) distributions for fertilizer-derived nitrate in the Webster soil

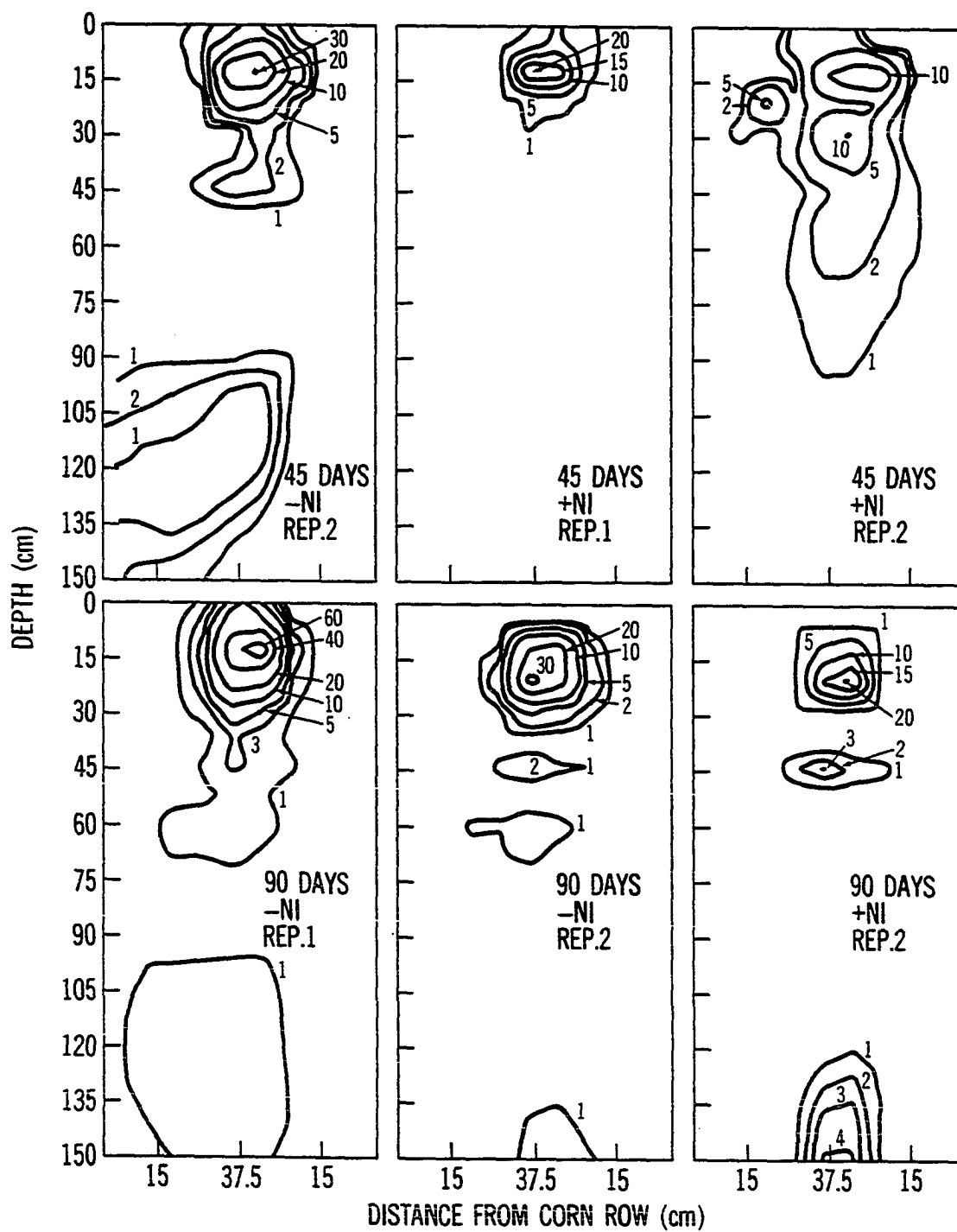
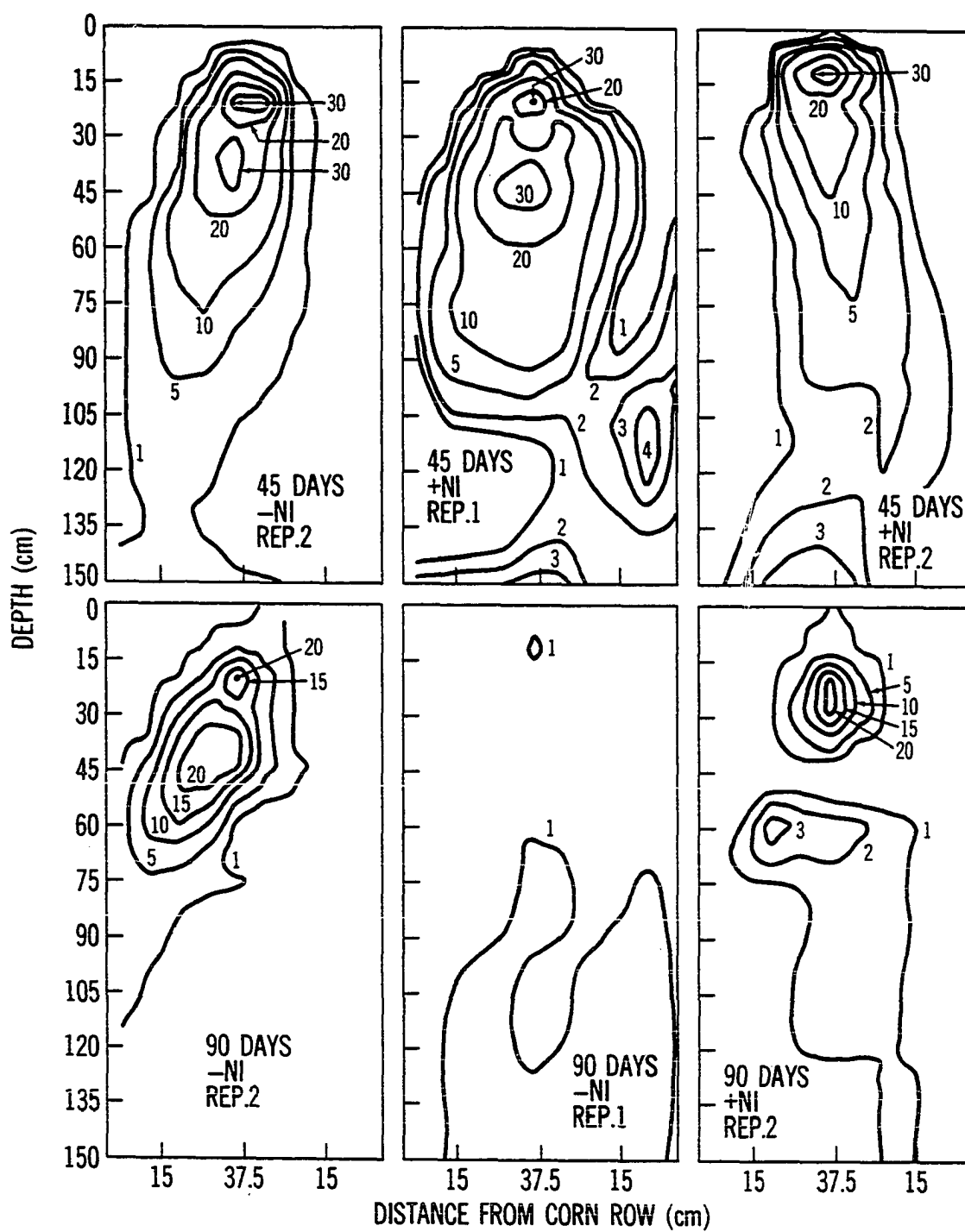


Figure 5. Concentration ( $\text{mg N Kg}^{-1}$  soil) distributions for fertilizer-derived nitrate in the Clarion soil



concentrations around fertilizer bands (Blue and Eno, 1954; McIntosh and Frederick, 1958; McDowell and Smith, 1958; Nommik, 1963a; Gasser and Ross, 1975). The Webster soil had higher concentrations of fertilizer-derived ammonium at 45 days than did the Clarion soil. This observation is consistent with the higher cation exchange capacity of the Webster soil. Comparisons of the isoconcentration lines for profiles collected at 45 and 90 days indicate that ammonium levels markedly decreased between these sampling dates.

The positions of the isoconcentration lines indicate that the concentrations of fertilizer-derived ammonium are better described as being log-normally distributed than as being normally distributed. This observation is important for two reasons. First, any statement concerning the diameter of the ammonium band has little meaning unless the concentration of ammonium designated as the outer boundary of the band is also defined. The diameter of these bands would have seemed much smaller if I had not used labeled fertilizer material, which allowed a separation between soil-derived ammonium and fertilizer-derived ammonium. Second, the determined values for concentrations of fertilizer-derived ammonium at locations near the point of injection will be extremely dependent on the exact locations at which the samples are taken. Therefore, large sampling errors must be expected from methods that sample only selected volumes of soil near the band (Hughes and Welch, 1970; Touchton et al., 1978).

Although the methods used in this study were designed to enable accurate determination of the amounts of fertilizer-derived ammonium-N

in the surface 37.5-cm layer, the positions of the isoconcentration lines were determined by a computer model. For this reason, I caution against comparing profiles for relatively small differences in concentrations near the point of application. With the exception of data collected at 45 days on the Clarion soil, the differences between profiles with nitrapyrin and profiles without nitrapyrin probably are not great enough to be considered significantly different in these figures.

Figures 4 and 5 show selected concentration distribution patterns for fertilizer-derived nitrate found in Webster and Clarion soils, respectively. The isoconcentration lines show that the fertilizer N moved downward from the point of injection and that this downward movement was primarily by convection rather than diffusion. Little lateral movement of N occurred, even though lateral movement was detectable in some profiles. In some profiles (e.g., Webster at 90 days with NI, Rep. 2), isolated areas of high nitrate concentrations occurred at various depths below the ammonia retention zone. Such a concentration distribution would be expected if nitrate was carried by water moving by displacement through the soil. In other profiles (e.g., Clarion at 45 days without NI, Rep. 2.), the concentration of fertilizer-derived nitrate decreased with depth in the soil. Such a concentration distribution would be expected if the nitrate was carried by water that moved preferentially through larger pores or cracks within the soil. The nitrate distributions of most profiles suggested that both displacement and preferential flow of water occurred. Even if the

mechanism of water movement is not considered, the nitrate distributions found at the greatest depths sampled suggest that some fertilizer-derived nitrate leached below the depth sampled.

It is important to recognize that the knives used to apply anhydrous ammonia leave planes of weakness in the soil that can be used as avenues for rapid water movement into the soil during intense rainfall events. Touchton et al. (1978) observed that soil within the retention zone was generally wetter than soil outside this zone and attributed this difference to faster water infiltration down the applicator knife tracks. Spatial variability in amounts of water that flow through the knife tracks as well as the influence of cracks, worm holes, and old root channels could be a major factor contributing to the variability in fertilizer recovery observed in this study. Other researchers (Chalk et al., 1975; Touchton et al., 1978; Hendrickson et al., 1978) observed similar variability.

The amounts of fertilizer-derived ammonium and nitrate found in the surface 150-cm layer of soils are shown in Figure 6. In the Webster soil, nitrapyrin had no apparent effect on the amounts of fertilizer-derived ammonium or nitrate found at 45 or 90 days. In the Clarion soil, nitrapyrin increased amounts of fertilizer-derived ammonium and decreased amounts of fertilizer-derived nitrate at 45 days. At 90 days in this soil, nitrapyrin had no effect on the amounts of fertilizer-derived ammonium but it substantially decreased the amounts of fertilizer-derived nitrate in both replications.

The data presented in Figure 6 also show that, especially in the

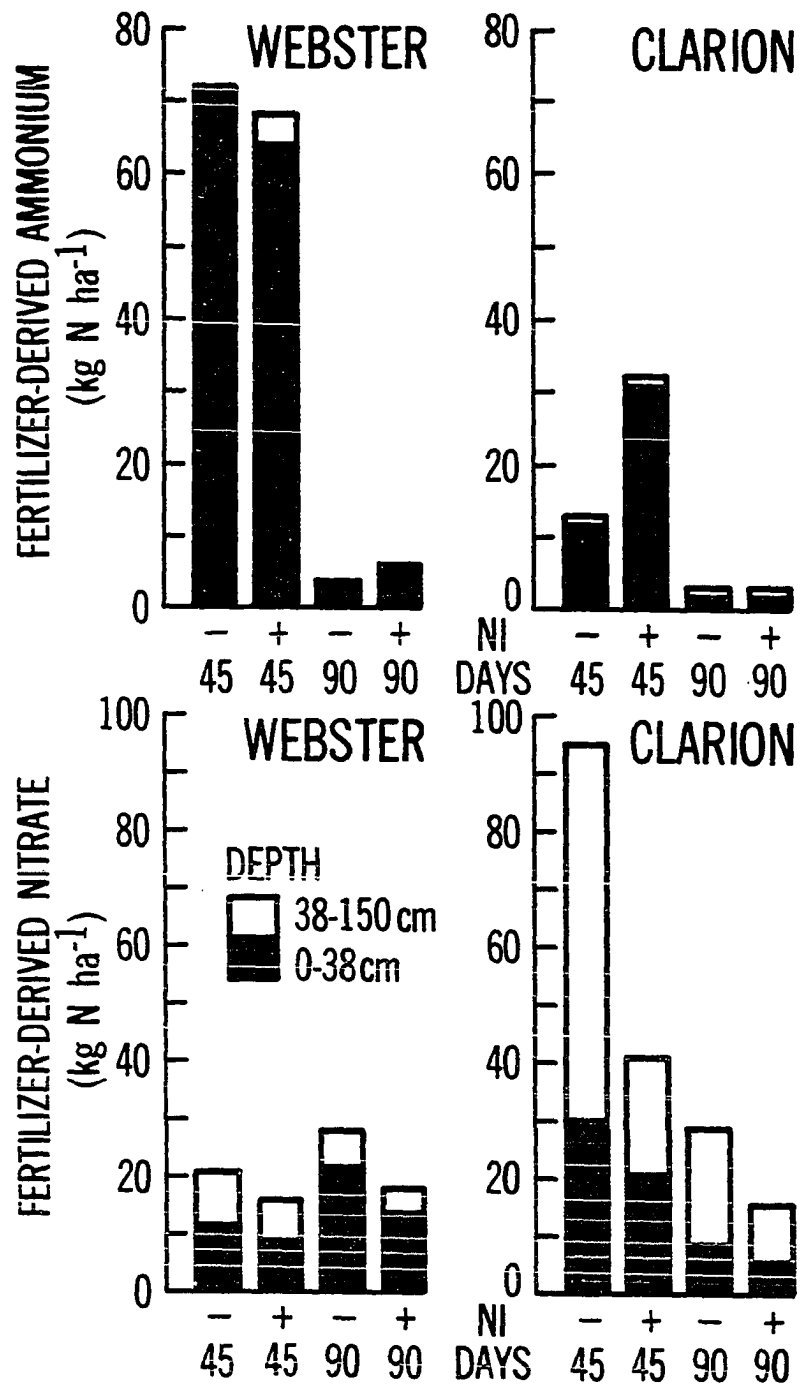


Figure 6. Amounts of fertilizer-derived ammonium and nitrate found in the Webster and Clarion soils



Clarion soil, a substantial portion of the fertilizer-derived nitrate was recovered below 37.5 cm. In most cases, the amounts of fertilizer-derived nitrate found within the ammonia retention zone represented only a small portion of the amounts of this N recovered to a depth of 150 cm. For example, in Rep. 1 at 45 days without nitrapyrin in the Clarion soil (Figure 4), the amount of fertilizer-derived nitrate within the ammonia retention zone represented only 10% of the fertilizer nitrate found. This finding is contrary to the assumption of Hendrickson et al. (1978) that information concerning the distribution of nitrate within the ammonia retention zone can be used to draw conclusions concerning the availability of fertilizer-derived nitrate to plants or the effectiveness of nitrification inhibitors. The data presented in Figure 6 also show the importance of  $^{15}\text{N}$  tracers in detecting small concentrations of nitrate in the soil (also see figures 4 and 5), which add up to significant quantities of fertilizer over the entire rooting volume.

The discussion up to this point has focused only on fertilizer-derived ammonium and nitrate. However, more than 50% of the mineral N (ammonium plus nitrate) present was derived from the soil at both sampling dates. Figure 7 shows the total amounts (fertilizer plus soil derived) of ammonium and nitrate found in the surface 150-cm layers of the two soils. There were no apparent differences due to nitrapyrin in the amounts of ammonium or nitrate found in the Webster soil at 45 days. This should be expected because, as discussed earlier, there were no apparent effects of nitrapyrin on the amounts of fertilizer-derived

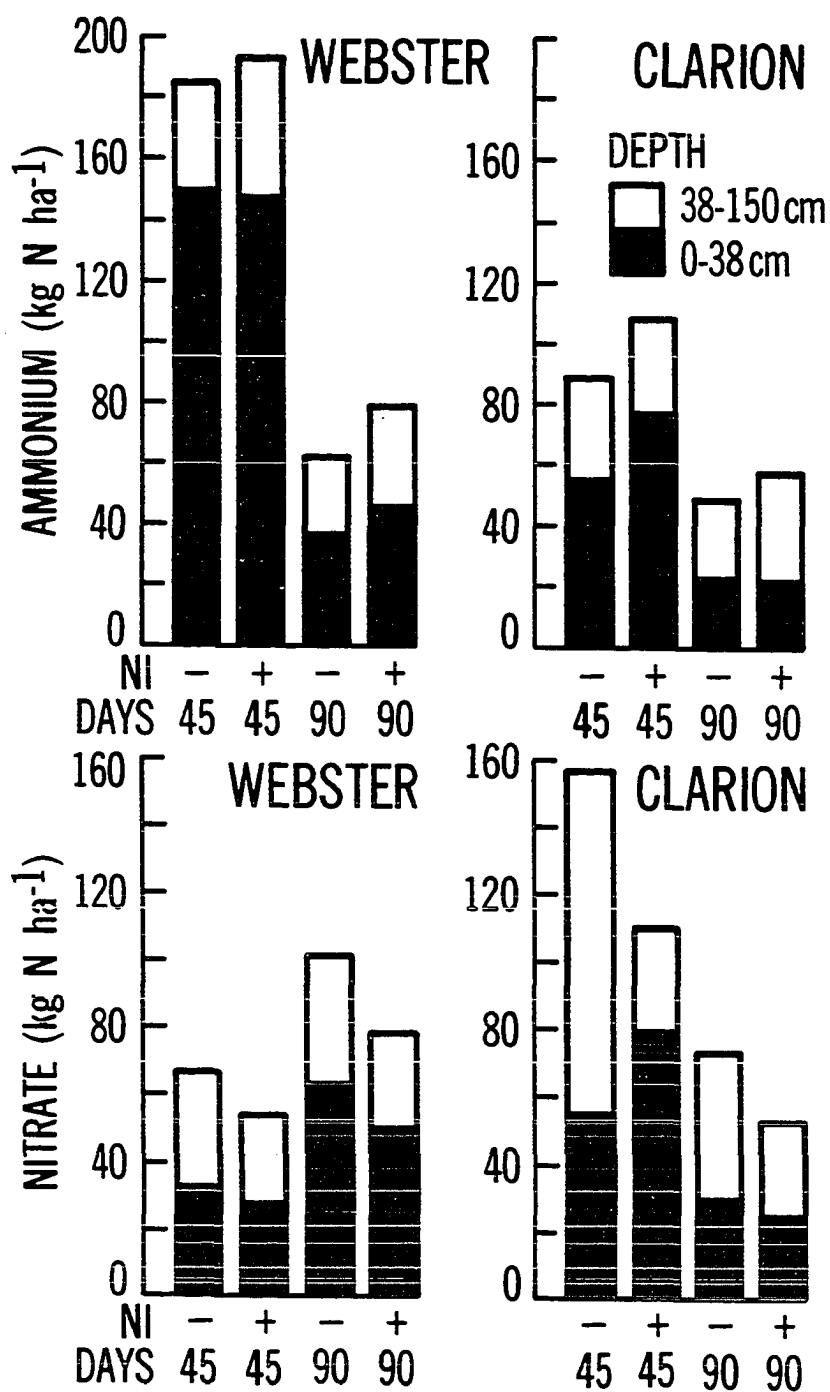
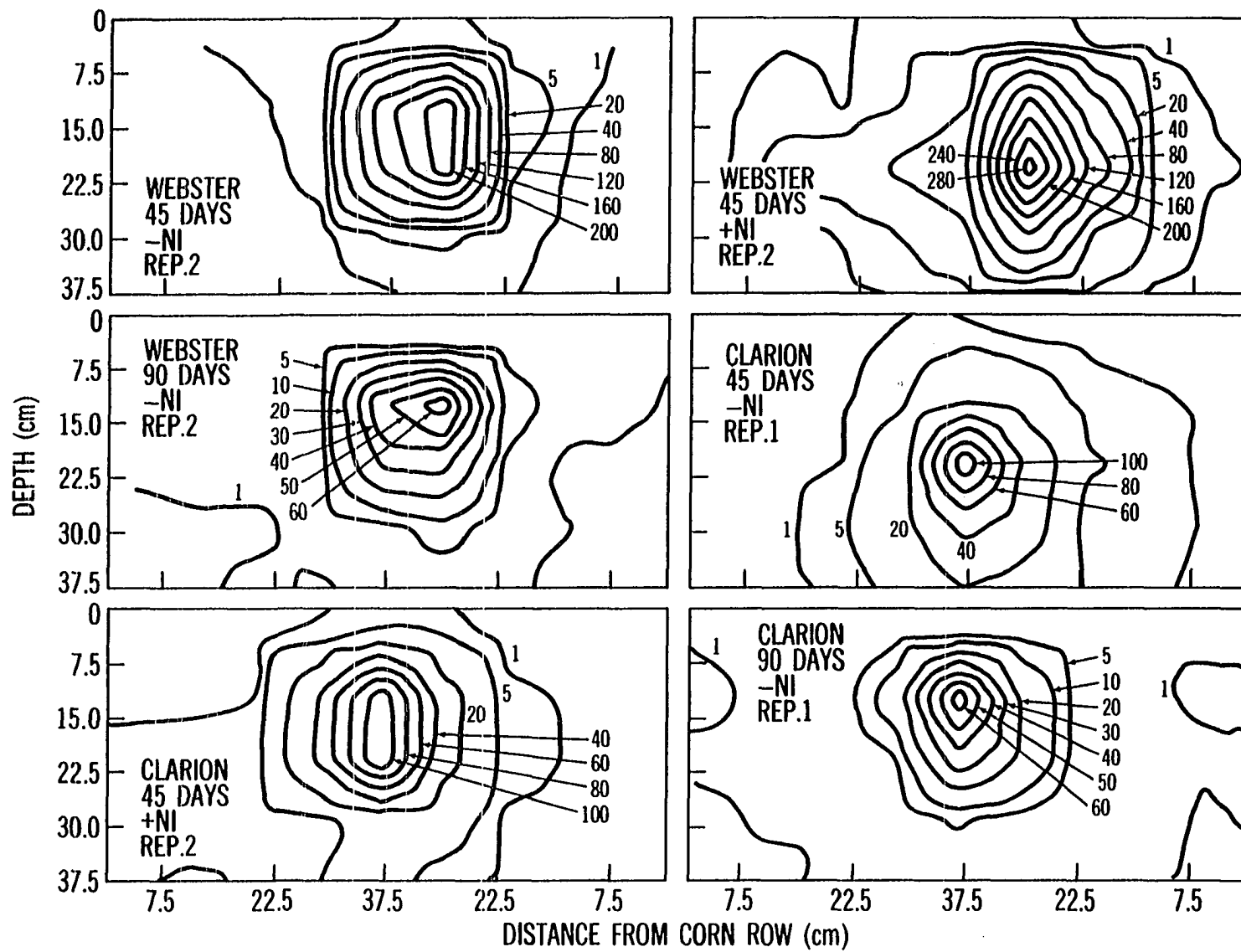


Figure 7. Amounts of ammonium and nitrate found in the Webster and Clarion soils

ammonium or nitrate N found in this soil. In the Clarion soil at 45 days, the effects of nitrapyrin on amounts of fertilizer-derived ammonium and nitrate are also reflected in the total amounts of ammonium and nitrate found. By 90 days in both soils, most of the ammonium and nitrate remaining in the soil profile was derived from the soil. Because of the large amounts of ammonium found in these soils (Figure 7), there probably is little practical benefit from using nitrification inhibitors to control the ratio of ammonium to nitrate in the applied fertilizer. Some researchers (Goring, 1962b) have suggested that such a practice could have beneficial effects on plant nutrition.

In samples collected near the point of fertilizer application, a large portion of the fertilizer-derived N recovered by Kjeldahl analysis was not accounted for as nitrate or exchangeable ammonium. Because this N could be in soil organic matter, microbial biomass, corn root residues, or nonexchangeable ammonium and because no attempt was made to distinguish between these forms of N, this N is referred to as KMI-N (Kjeldahl minus inorganic) in this report. The amount of N in root residues may be important because a very dense proliferation of fine roots was observed during excavation of soil profiles at both sampling dates and no attempt was made to separate fine roots from the soil. The concentration distribution for KMI-N in selected profiles is shown in Figure 8. Because the concentration distributions of the fertilizer-derived KMI-N fraction showed less variability than did the concentration distributions of fertilizer-derived nitrate or ammonium, data for fewer profiles are presented.

Figure 8. Concentration ( $\text{mg N Kg}^{-1}$  soil) distributions for fertilizer-derived N in the KMI-N fraction



The amounts of fertilizer-derived N recovered as nitrate, ammonium, and KMI-N from profiles with and without nitrapyrin are shown in Table 2. Data presented show that, in the absence of nitrapyrin, about 80 and 70% of the fertilizer N could be accounted for at 45 days in the Webster and Clarion soils, respectively. The KMI-N fraction accounted for 41% of the fertilizer N recovered at 45 days. Because only 49% of the N in this fraction was still present at 90 days, much of the N in this fraction became available to the plants during the growing season. The observation that large amounts of the ammonia applied were found in non-exchangeable forms is consistent with the results of laboratory studies reported by others (Sohn and Peech, 1958; Young, 1964).

Differences among treatments were not large enough to make reliable conclusions concerning the effects of nitrapyrin on amounts of N recovered in the KMI-N fraction. Data presented in Part IV show that nitrapyrin had no consistent effect on the amounts of N recovered as KMI-N one, two and three years after fertilization. Overall, at 45 days, nitrapyrin had no effect on recovery of fertilizer N in the Webster soil and decreased recovery of fertilizer N in the Clarion soil. At 90 days, nitrapyrin had no effect on recovery of fertilizer N in either soil. Many workers have reported that nitrification is inhibited in ammonia retention zones (Eno and Blue, 1954; Eno et al., 1955; Stevens and Reuss, 1975; Khengre and Savant, 1977) and there may be little practical benefit from the use of nitrification inhibitors with anhydrous ammonia under many conditions.

A possible explanation as to how nitrapyrin decreased recovery of

Figure 9. Distributions of pH found in the Webster and Clarion soils

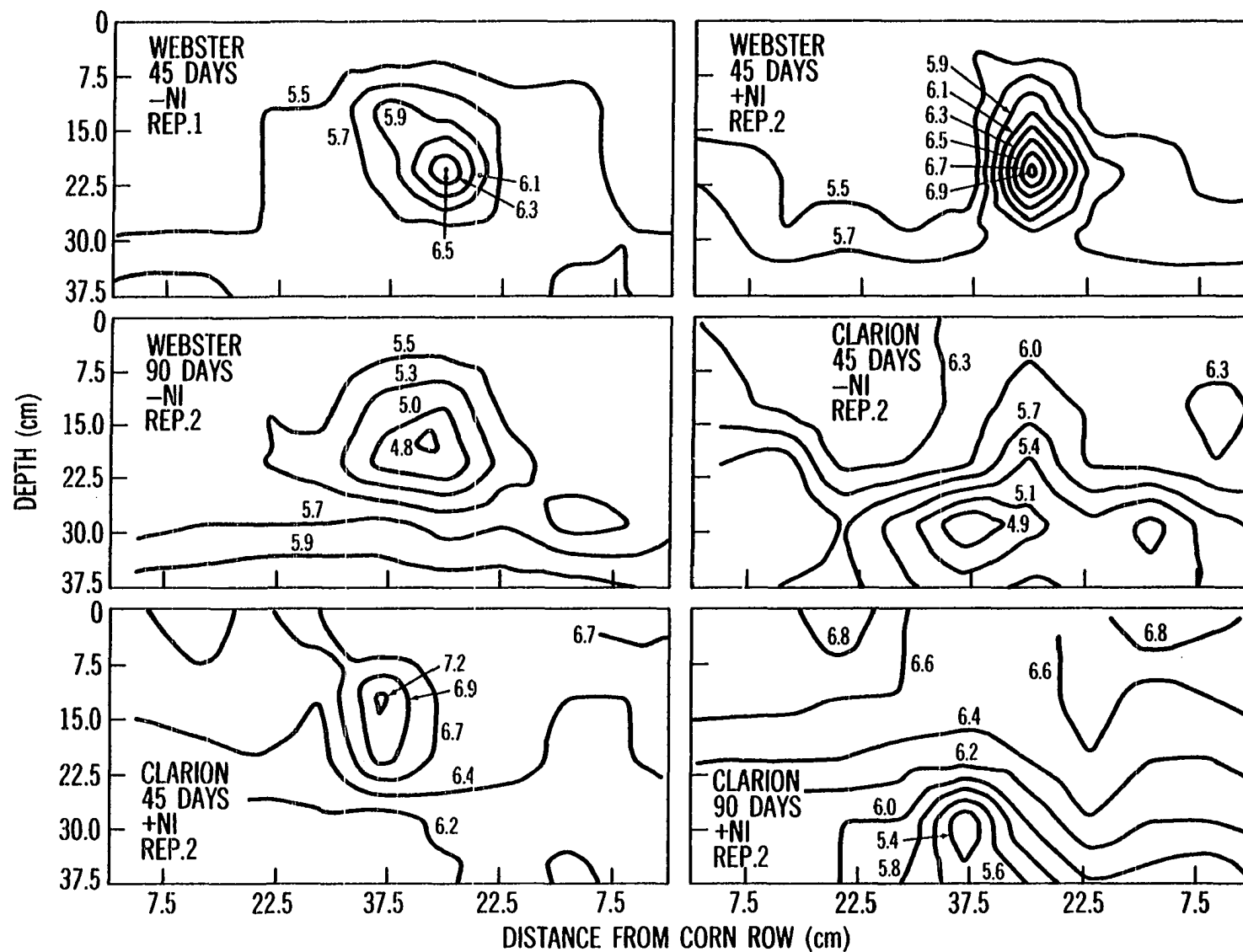




Table 2. Recovery of anhydrous ammonia-derived N in various fractions at 45 and 90 days after fertilization

Soil	NI	Amount recovered				
		NH <sub>4</sub> -N	NO <sub>3</sub> -N	KMI-N	Plant-N	Total-N
-----Kg ha <sup>-1</sup> -----						
<u>45 Days</u>						
Webster	-	72	21	84	5	182
	+	68	16	90	5	179
Clarion	-	13	96	40	5	154
	+	32	42	52	5	131
<u>90 Days</u>						
Webster	-	4	28	42	66	140
	+	6	18	38	77	139
Clarion	-	3	29	28	58	118
	+	3	16	18	74	111

fertilizer N in the Clarion soil at 45 days is provided by Figure 9, which shows the distribution of values for soil pH in selected profiles. Some profiles show the high pH values that should be expected (Blue and Eno, 1954; McIntosh and Frederick, 1958;) near the point of ammonia injection soon after application. Some profiles show the net acidification that should be expected after nitrification is complete. In the Clarion soil at 45 days, the pH in the ammonia retention zone with nitrapyrin was about 3 pH units higher than without nitrapyrin. Such a difference should be expected from a nitrification inhibitor. Because nitrapyrin prolonged the existence of the high pH zone in this soil, and because high soil pH promotes losses of N by ammonia volatilization, it is possible that the addition of nitrapyrin induced losses of fertilizer N by this mechanism. If ammonia volatilization was a factor, the differences between soils in effect of nitrapyrin on ammonia volatilization could be explained by the lower initial pH and higher CEC (buffering capacity) of the Webster soil. Also, nitrapyrin had less effect on nitrification in this soil.

The data presented in this report indicate that the total amounts of fertilizer-derived N in the rooting zone of plants must be considered when assessing rates of nitrification, availability of fertilizer N to plants, or effects of nitrification inhibitors on this availability. Measurements of the amounts of nitrate within only the ammonia retention zone often represent a only small fraction of the nitrate within the rooting zone of corn. Measurements of only ammonium and nitrate exclude that proportion of the fertilizer N that moves into the KMI-N fraction, some of which is released during the growing season.

## SUMMARY

The distribution and recovery of anhydrous ammonia-derived N within the rooting zone of corn was studied by intensively sampling the 150-cm layer of two soils during the growing season. The results showed that fertilizer-derived ammonium moved little from the point of ammonia injection. However, fertilizer-derived nitrate moved downward rapidly, and leaching was probably an important mechanism of N loss. In most profiles, only a small fraction of the fertilizer-derived nitrate N that was recovered within the rooting zone was located within the ammonia retention zone.

At 45 days after application, an average of 75% of the fertilizer N was recovered when nitrapyrin was not applied and 70% when nitrapyrin was applied. At 90 days, an average of 58% of the fertilizer N was recovered when nitrapyrin was not applied and 55% when nitrapyrin was applied. About 41% of the fertilizer N recovered by Kjeldahl analysis at 45 days could not be accounted for as exchangeable ammonium or nitrate. Because only 59% of the N in this KMI-N fraction was still present at 90 days, much of the N in this fraction became available to the plants during the growing season.

These findings indicate that the customary practice of measuring ammonium and nitrate levels from only within the ammonia retention zone may provide unreliable assessments of nitrification rates in soils, availability of fertilizer N to plants, or effect of nitrification inhibitors on this availability.

PART VI. EFFECTS OF NITRAPYRIN ON RECOVERY OF NITRATE  $^{15}\text{N}$   
IN SOIL-PLANT SYSTEMS.

## INTRODUCTION

Mills and Pokorny (1978) and McElhannon and Mills (1981) recently reported that nitrapyrin performed a dual role in crop production by inhibiting both nitrification and denitrification of fertilizer N in soils. The ability of nitrapyrin to inhibit nitrification has been well established (Goring, 1962a,b; Keeney, 1980; Meisinger et al., 1980; Hauck, 1983), and it is generally accepted that this compound can be used to conserve N from ammonium or ammonium-yielding fertilizers under conditions that favor leaching or denitrification of nitrate. It also has been suggested (Goring, 1962a) that this compound can be used to alter the ratio of ammonium to nitrate in soils and thereby provide benefits from a standpoint of plant nutrition.

The reports that nitrapyrin has a significant inhibitory effect on denitrification in soils is not supported by reports (Goring, 1962a; Shattuck and Alexander, 1963; and Tu, 1973) that the inhibitory effect of nitrapyrin is highly specific for nitrifying microorganisms and has little effect on other soil microorganisms. Furthermore, it is not supported by the studies of Mitsui et al. (1964), Henninger and Bollag (1976), or Bremner and Blackmer (1980), which show that nitrapyrin has little or no effect on denitrification in soils. However, all of these studies were conducted in soils without plants. Because Cribbs and Mills (1979) reported that the presence of a plant was necessary for nitrapyrin to be an effective inhibitor of denitrification, it could be argued that studies conducted without plants cannot be used to evaluate

the reports of Mills and Pokorny (1978) and McElhannon and Mills (1981).

The conclusion of Mills and Pokorny (1978) and McElhannon and Mills (1981) that nitrapyrin has a significant inhibitory effect on denitrifying microorganisms was based largely on their observations that, even when applied with nitrate fertilizers, nitrapyrin increased plant growth and recovery of fertilizer N in plants and soils or growth media. The studies reported here were conducted to determine if these effects of nitrapyrin could be observed in soil-plant systems in which <sup>15</sup>N-tracers were used to facilitate determinations of N recovery.

## MATERIALS AND METHODS

Pots were prepared in a full factorial design replicated five times with two soils and four rates of added nitrate (0, 0.5, 1.0, and 1.5 g nitrate-N  $\text{pot}^{-1}$ ) with and without nitrapyrin. The soils used were a clay loam  $A_p$  horizon collected from a Webster (fine-loamy, mixed, mesic Typic Haplaquoll) mapping unit and a clay loam  $A_p$  horizon collected from a Harps (fine-loamy, mesic, Typic Calciaquoll) mapping unit. Some properties of these soils are shown in Table 1.

Each pot contained 9.15 kg air-dried soil that had been passed through a 4-mm sieve. The soil from each pot was spread over a  $1\text{-m}^2$  sheet of plastic for application of the fertilizer treatments. Nitrate and nitrapyrin treatments were applied as aqueous solutions that were sprayed uniformly over the soils. These solutions were prepared by mixing various amounts (0, 30, 60, and 90 ml) of a solution containing  $^{15}\text{N}$ -labeled nitrate (16.5 mg nitrate-N  $\text{ml}^{-1}$  as calcium nitrate and 0.4 mg nitrate-N  $\text{ml}^{-1}$  as potassium nitrate having 99 atom percent  $^{15}\text{N}$ ) with various amounts (0 or 10 ml) of a solution containing nitrapyrin (1 ml of N-Serve 24E in 847 ml of water) and adding enough distilled water to make a total volume of 200 ml. The equivalent of 56 kg P  $\text{ha}^{-1}$  as triple superphosphate and 168 kg K  $\text{ha}^{-1}$  as potassium was spread on the soil from each pot. The soil for each pot was then mixed in a mechanical mixer and returned to the pots. The N treatments were equivalent to 0, 100, 200, or 300 kg N  $\text{ha}^{-1}$ , with and without 2.3 L of N-Serve 24E  $\text{ha}^{-1}$ .

Each pot was planted (December 1) with eight seeds of corn (SAR

Table 1. Selected properties of soils used in study

Soil	pH	CaCO <sub>3</sub> equivalent	Sand	Clay	Organic C	Total N	Exchangeable ammonium	Nitrate
		-----%-----			----mg g <sup>-1</sup> ----		-----mg N kg <sup>-1</sup> -----	
Webster	6.6	0	32	28	27	1.7	8.8	18.1
Harps	8.0	10	15	38	46	3.2	5.8	11.0



SX4900) and the resulting seedlings were thinned to one plant per pot. Water was periodically added to bring the soils near the point of saturation. The quantities of water added were carefully controlled by monitoring total pot weights. To avoid losses of nitrate by leaching, the holes on the bottoms of each pot were sealed with water proof tape and each pot was set within an aluminum pan to retain any leachate. Periodically, the pans were rinsed into the appropriate pots. Corn shoots and roots were harvested at tasseling (March 12), dried at 65°C for 36 hours, and ground in a Cyclone mill. Roots from each pot were harvested by sieving (2-mm screen) the soil. The soil within each pot was thoroughly mixed and samples were collected for analyses.

Exchangeable ammonium-N and (nitrate plus nitrite)-N contents of the soils were determined by extraction with 2 N KCl and steam distillation with magnesium oxide and Devarda alloy as described by Keeney and Nelson (1982). Because distillates from these analyses were used for  $^{15}\text{N}$  determinations, 5 ml of an ammonium nitrate standard containing 15 ug ammonium-N  $\text{ml}^{-1}$  and 15 ug nitrate-N  $\text{ml}^{-1}$  were added to each aliquot (20 ml) of soil extract distilled. This practice assured that each sample contained enough N to be within the working range of the mass spectrometer used for  $^{15}\text{N}$  determinations. To avoid cross contamination of samples by ammonium exchange processes on the condenser (see Hauck, 1982), separate distillation systems were used for ammonium-N and (nitrate plus nitrite)-N distillations and duplicate aliquots of each soil extract were analyzed. Distillates from the first aliquots were collected in boric acid indicator solution and then titrated with

acid as described by Keeney and Nelson (1982). Distillates from the second aliquots were collected in 2 ml of 0.08  $\underline{\text{N}}$   $\text{H}_2\text{SO}_4$ , concentrated (by evaporation of water) to a volume of about 2 ml, and stored in 2-dram vials.

Total N contents of the soils and plant residues were determined by the permanganate-reduced iron modification of the Kjeldahl procedure as described by Bremner and Mulvaney (1982). Following titration with 0.05  $\underline{\text{N}}$   $\text{H}_2\text{SO}_4$ , each distillate was acidified by addition of 2 ml of 0.08  $\underline{\text{N}}$   $\text{H}_2\text{SO}_4$ , concentrated to a volume of about 2 ml, and stored in a 2-dram vial.

Determinations of  $^{15}\text{N}$  in soils, soil extracts, and plant residues were performed by reacting the concentrated distillates with sodium hypobromite in evacuated Rittenberg flasks as described by Hauck (1982) and injecting the resulting dinitrogen gas into a Varian MAT 250 mass spectrometer.

## RESULTS AND DISCUSSION

Table 2 shows the effects of nitrapyrin and rate of nitrate application on dry matter yield, N concentration in plant tissue, N uptake, and the percentage of N in the plant derived from fertilizer in two soils. Data presented suggest that nitrapyrin had no effect on plant growth or uptake of N. An analysis of variance indicated that significant effects of nitrapyrin were not detectable even at the 10% level of probability. Dry matter yields, N concentration in plant tissue, and N uptake significantly ( $P > 0.01$ ) increased with amount of applied nitrate. I consider N concentration in plant tissue and N uptake to be reliable indicators of N availability to plants because both increased with each additional increment of added nitrate.

Table 3 shows the effects of nitrapyrin and rate of nitrate application on the concentrations of nitrate-N and exchangeable ammonium-N remaining in the soil following plant harvest. Analysis of variance showed that nitrapyrin had no effect on the concentrations of nitrate or ammonium remaining in the soil following harvest.

Table 4 shows effects of nitrapyrin and rate of nitrate application on the amounts of fertilizer-derived N found in nitrate, soil organic matter, plant shoots, and plant roots in the two soils. Analysis of variance showed that nitrapyrin had no significant effect on the amount of N in any of these fractions. Isotope ratio analyses revealed that negligible amounts of fertilizer-derived N were present as exchangeable ammonium. The total amounts of fertilizer-derived N found indicate that

Table 2. The effects of nitrification inhibitor and rate of nitrate application on dry matter yield, N concentration in plant tissue, N uptake, and the percentage of plant N derived from fertilizer in two soils

Soil	Rate of N application	NI	Dry matter yield	Concentration in tissue	N uptake	Percentage of plant N derived from fertilizer
	g N pot <sup>-1</sup>		g pot <sup>-1</sup>	mg g <sup>-1</sup>	g pot <sup>-1</sup>	%
Webster	0	-	28.2	8.4	0.24	0
	0	+	29.9	8.8	0.26	0
	0.506	-	38.8	16.0	0.61	50
	0.506	+	39.8	17.0	0.67	52
	1.012	-	38.0	21.9	0.84	68
	1.012	+	40.2	19.9	0.79	68
	1.518	-	37.9	21.9	0.82	76
	1.518	+	40.7	20.1	0.82	74
Harps	0	-	36.3	9.6	0.34	0
	0	+	35.2	8.8	0.31	0
	0.506	-	38.9	17.1	0.66	47
	0.506	+	35.1	18.2	0.64	45
	1.012	-	36.2	21.4	0.78	61
	1.012	+	34.7	20.8	0.72	63
	1.518	-	32.8	21.7	0.71	68
	1.518	+	35.7	22.1	0.79	70

Table 3. The effects of nitrification inhibitor and rate of nitrate application on the concentrations of nitrate-N and ammonium-N remaining in the soil following plant harvest

Soil	Rate of N application	NI	Concentration	
			Nitrate	Ammonium
	g N pot <sup>-1</sup>		--mg N kg soil <sup>-1</sup> --	
Webster	0	-	5	6
	0	+	7	5
	0.506	-	9	4
	0.506	+	9	5
	1.012	-	47	5
	1.012	+	45	6
	1.523	-	89	5
	1.523	+	82	6
Harps	0	-	7	5
	0	+	7	4
	0.506	-	13	5
	0.506	+	19	5
	1.012	-	56	4
	1.012	+	63	4
	1.523	-	105	6
	1.523	+	108	3

Table 4. The effects of nitrification inhibitor and rate of nitrate application on the amounts of fertilizer-derived N found in nitrate, soil organic matter, plant shoots, and plant roots in two soils

Soil	Rate of N appli- cation	NI	Amounts of fertilizer-derived N found				
			In $\text{NO}_3^-$	In SOM	In roots	In shoots	Total
	g N $\text{pot}^{-1}$		-----g $\text{pot}^{-1}$ -----				
Webster	0.506	-	0.03	0.08	0.03	0.30	0.44
	0.506	+	0.02	0.07	0.02	0.36	0.46
	1.012	-	0.23	0.10	0.05	0.56	0.95
	1.012	+	0.24	0.10	0.05	0.54	0.92
	1.518	-	0.57	0.09	0.08	0.62	1.35
	1.518	+	0.52	0.13	0.09	0.61	1.35
Harps	0.506	-	0.03	0.07	0.04	0.31	0.45
	0.506	+	0.05	0.10	0.04	0.29	0.49
	1.012	-	0.28	0.12	0.07	0.47	0.94
	1.012	+	0.35	0.09	0.06	0.46	0.95
	1.518	-	0.69	0.17	0.07	0.49	1.42
	1.518	+	0.72	0.14	0.07	0.56	1.49

only 5 to 11% of the added nitrate was lost from the soil-plant systems and, therefore, that denitrification of added nitrate could not have been a major factor affecting availability of N to plants. This finding is consistent with the results presented in Tables 2 and 3. It was unexpected because the soils were maintained at soil moisture levels intended to promote losses of N by denitrification. Several studies (Blackmer and Bremner, 1977; 1978; Gaskell et al., 1981; Patten et al., 1980) have shown that the Harps and Webster soils have a high capacity for denitrification under anaerobic conditions.

Because nitrapyrin did not alter the percentage of plant N derived from fertilizer, the yields of plants, the amounts of N in the plants, or the amounts of nitrate found in the soil after plant harvest, this compound could not have inhibited the denitrification of significant amounts of soil-derived nitrate. Also, it could not have enhanced plant growth by providing a more favorable ratio of ammonium to nitrate.

The finding that denitrification was not a major factor affecting nitrate availability in these studies prompted me to reevaluate the evidence for significant amounts of denitrification in the studies of Mills and Pokorny (1978), Cribbs and Mills (1979), and McElhannon and Mills (1981). I found that Mills and Pokorny (1978) presented no unequivocal evidence that significant amounts of denitrification occurred. Their conclusion that nitrapyrin inhibited denitrification was based on the observations that nitrapyrin increased amounts of nitrate found in a nitrate-treated sand-bark media, increased growth of plants in this media, and increased amounts of N taken up by these

plants. These observations could be explained by the combined effects of a nitrification inhibitor and losses of N by leaching. A possible explanation is as follows. All pots containing the sand-bark media were treated with Hoagland's solution for 2 weeks before nitrapyrin was added. I suspect that ammonium from this solution remained in the soil when the nitrate and nitrapyrin treatments were applied and that the addition of nitrapyrin delayed conversion of this N to nitrate. Because the authors indicated that preliminary studies showed as much as 30% of the applied ammonium was lost by leaching, it is evident that nitrate was lost by leaching. In the presence of leaching, a delay in nitrification of the residual ammonium should be expected to increase the availability of N to plants. If, as observed by Mills and Pokorny (1978), the inhibitory effect of nitrapyrin on nitrification decreases with time, then addition of a nitrification inhibitor should be expected to increase nitrate levels in the medium for a period after inhibition of nitrification diminishes.

Cribbs and Mills (1979) measured nitrous oxide evolution from an organic growth medium to show that denitrification occurred. However, these measurements do not provide unequivocal evidence that denitrification was an important mechanism of N loss because the amounts of nitrous oxide evolved represented less than 1% of the added nitrate N. McElhannon and Mills (1981) measured emissions of nitrous oxide from soils under field conditions at selected times, but they did not present information showing that the amounts of nitrous oxide evolved from their soils represented a significant reduction in plant-available N. Many



studies (Breitenbeck et al., 1980; Mosier and Hutchinson, 1981; Conrad et al., 1983) have reported that emissions of nitrous oxide under field conditions are small compared to the amounts of fertilizer usually applied for crop production.

The conclusion of McElhannon and Mills (1981) that nitrapyrin inhibited denitrification was based on the observations that, when added with nitrate, nitrapyrin increased amounts of nitrate found in the soil, increased yields of plants (corn) growing in this soil, and increased amounts of N taken up by these plants. These authors presented no evidence to indicate that these effects of nitrapyrin could not be caused by the combined effects of leaching and a nitrification inhibitor, which can delay nitrification of soil-derived ammonium.

The conclusion of Cribbs and Mills (1979) that nitrapyrin had a significant inhibitory effect on denitrification in plant growth media originates from the observation that nitrapyrin decreased the rate of nitrous oxide production during the seventh and eighth days of an eight-day study. A corresponding decrease in rate of nitrous oxide production did not occur when nitrapyrin was not added. The validity of this conclusion can be questioned because nitrapyrin significantly increased rates of nitrous oxide evolution for the first five days and because the amounts of nitrous oxide evolved during the 8-day period were similar with and without nitrapyrin. Also, their results showed that nitrapyrin greatly increased rates of nitrous oxide emission from growth media without plants.

The studies reported here do not prove that denitrification was not

a major factor limiting the availability of N in the studies of Mills and Pokorny (1978) or those of McElhannon and Mills (1981). Furthermore, they do not prove that nitrapyrin cannot inhibit denitrification in soil-plant systems. However, our observation that only small amounts of denitrification could have occurred in soil-plant systems that were maintained near the point of saturation suggests that any inhibitory effect of nitrapyrin on denitrification may be of little practical importance for crop production under many conditions. These results also emphasize the need for careful resolution of leaching and denitrification as mechanisms of N loss when evaluating the ability of a nitrification inhibitor to inhibit denitrification in soil-plant systems.

## SUMMARY

<sup>15</sup>N tracers were used to study the effects of nitrapyrin (a nitrification inhibitor) on recovery of nitrate in soil-plant systems having soil moisture levels intended to promote denitrification. The studies were prompted by recent reports that nitrapyrin has a significant inhibitory effect on denitrification in soils when plants are present. These reports were based largely on the observations that, even when applied with nitrate fertilizers, nitrapyrin increased plant growth and recovery of fertilizer N in plants and soil.

Although the soils in this study were maintained at moisture levels intended to promote denitrification, little denitrification occurred and nitrapyrin had no significant effects on growth of corn (Zea mays L.) or recovery of N. These findings suggest that any inhibitory effect of nitrapyrin on denitrification may be of little practical importance during crop production under many conditions. The reports that nitrapyrin inhibits denitrification in soils with plants may be the result of failure to consider the effects of nitrapyrin on nitrification of soil-derived N and failure to distinguish between leaching and denitrification as mechanisms of N loss.

## GENERAL SUMMARY

Field studies were conducted for three years at two sites in Iowa to acquire a better understanding of the transformations and movement of anhydrous ammonia-derived N in soils and the response of corn to this N. Because nitrification inhibitors have been widely recognized as having potential for improving the efficiency of N fertilization, the effects of nitrapyrin (the most widely used nitrification inhibitor) on the transformations and movement of fertilizer N were also studied.

In Part I, a method is described that permits precise application of anhydrous ammonia in bands to plots of the size often used in  $^{15}\text{N}$ -tracer studies. The procedure has marked advantages over previously described methods because it can be used with mixtures of anhydrous ammonia and nitrification inhibitors and because the soil environment at the point of application is representative of the soil environment found when a conventional applicator is used.

The research described in Part II was initiated to assess the importance of lateral movement of labeled fertilizer when unconfined plots are used to determine recovery of fertilizer. The results of these studies indicate that  $^{15}\text{N}$  plots having a size of 2 m by 2 m are sufficiently large for determining recovery of fertilizer N for corn crops under most soil conditions.

In Part III, the response of corn to anhydrous ammonia applied at 112 and 224 kg N ha<sup>-1</sup> with and without nitrapyrin was studied. Significant increases in grain yields were observed in response to N at

both sites, but this response was only to the first increment at one site. Nitrapyrin had a statistically significant effect on grain yields at only one of the 12 rate-site years, and this effect was negative. An abundance of fertilizer-derived and soil-derived N must be considered a major reason for lack of yield responses to nitrapyrin. However, integrated analyses of the results of determinations of N content, percentage of N derived from fertilizer, and dry matter yields for various plant parts suggest that nitrapyrin sometimes had adverse effects on plant growth. The adverse effects may have been the result of nitrapyrin increasing the susceptibility of plants to moisture stress.

In Part IV, studies were conducted at two locations to determine recovery of labeled anhydrous ammonia with and without nitrapyrin during corn production over a three-year period. The results showed that 13 to 33% of the labeled N was removed from the plots during corn harvest in the first crop after fertilization. Although the total amounts of labeled N found in the whole plants ranged from 29 to 45%, the stover was returned to the plots. Nitrapyrin increased the amount of N recovered in plant tissue at only one of the six site-years studied. Only small percentages (0.3 to 1.5%) of the labeled N were recovered in the second and third crops after fertilization. Nitrapyrin had negligible effects on the amounts of labeled N recovered by plants in the second and third growing seasons. These findings suggest that fertilizer N applied for one cropping season has little residual value in subsequent cropping seasons where fertilizers are applied each year.

Analyses of soil samples collected one year after fertilization showed that about 19 to 23% of the fertilizer N remained in the soil. Only small portions of this N were as exchangeable ammonium and nitrate. Most of this N was in the KMI-N (kjeldahl minus inorganic) fraction, which includes N from both organic matter and nonexchangeable ammonium. Large portions (47 to 94%) of the labeled N that were found in the KMI-N fraction one year after fertilization were still present three years after fertilization. The results of this study showed that a substantial portion (49 to 64%) of the labeled N was lost from the surface 1.5 m of soil during the first year by processes other than plant uptake.

In Part V, the distribution and recovery of anhydrous ammonia-derived N within the rooting zone of corn was studied by intensively sampling the 150-cm layer of two soils during the growing season. The results showed that fertilizer-derived ammonium moved little from the point of ammonia injection. However, fertilizer-derived nitrate moved downward rapidly, and leaching was probably an important mechanism of N loss.

At 45 days after application, an average of 75% of the fertilizer N was recovered when nitrapyrin was not applied and 70% when nitrapyrin was applied. At 90 days, an average of 58% of the fertilizer N was recovered when nitrapyrin was not applied and 55% when nitrapyrin was applied. About 41% of the fertilizer N recovered by Kjeldahl analysis at 45 days could not be accounted for as exchangeable ammonium or nitrate. Because only 59% of the N in this KMI-N fraction was still present at 90 days, much of the N in this fraction became available to

the plants during the growing season.

The effects of nitrapyrin on denitrification of nitrate are studied in Part VI. The studies were prompted by recent reports that nitrapyrin has a significant inhibitory effect on denitrification in soils when plants are present. The results obtained suggest that any inhibitory effect of nitrapyrin on denitrification may be of little practical importance during crop production under many conditions.

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