

DEVELOPMENT OF FIELD STANDARDS FOR
AN OPTICAL SENSOR VARIABLE RATE
APPLICATOR

ESTIMATING GASEOUS NITROGEN LOSSES
FROM *TRITICUM AESTIVUM* L.
USING ^{15}N

By

HEATHER LYNN LEES

Bachelor of Science

Wilmington College

Wilmington, Ohio

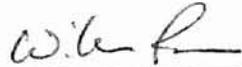
1995

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 1997

I. DEVELOPMENT OF FIELD STANDARDS FOR
AN OPTICAL SENSOR VARIABLE RATE
APPLICATOR

II. ESTIMATING GASEOUS NITROGEN LOSSES
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Thesis Approved:



Thesis Advisor



Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to express my thanks to the Agronomy Department of Oklahoma State University for giving me the opportunity to pursue a Master's Degree. A sincere thanks goes to my major advisor Dr. William R. Raun for his leadership, guidance, encouragement, and understanding. I would also like to extend my gratitude to the many members of the OSU Soil Fertility project, including: Hasil Sembiring, Jing Chen, Shannon Taylor, Steve Taylor, Steven Philips, Jeremy Dennis, Doug Cossey, Dale Keahey, Edgar Ascencio, Fred Kanampiu, Jerry Moore, Wade Thomason, Erna Lukina, Joanne LaRuffa, Darin Drury, Shawn Norton, Tina Johnson, Curt Woolfolk, Kathy Wynn and Ryan Miller. Also to the Biosystems and Agricultural Engineering Department including: Dr. Marvin Stone, Dr. Richard Whitney, John Ringer, Byron Criner, and Chad Dow. And to the members of my committee, Dr. Gordon Johnson and Dr. John Solie for their support and revisions. I would also like to thank Dr. Al Conklin for encouraging me to apply to Oklahoma State University.

Finally, I would like to recognize my parents David and Suzanne, my brothers Eric and Kent, and boyfriend, Shawn Burden for their constant encouragement and support.

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I. Development of Field Standards for Variable Rate Technology

Introduction

Variable rate technology (VRT) can be used to accurately assess and correct nitrogen (N) needs in winter wheat. VRT is a practice whereby fertilizer application over a field is changed in accordance with predetermined soil nutrient levels. VRT is a useful concept in U.S. agriculture because it proposes to apply only the amount of N needed by the crop thereby protecting not only the environment but also the producer's profit. VRT works because fields are not uniform. Therefore, when production inputs are applied based on soil test values from four soil samples of a ten hectare area, maximum profit does not occur (Sawyer, 1994). Hence, VRT enables the producer to capitalize on the variability within fields.

Measures of crop spectral reflectance that relate to corresponding N uptake may be utilized in variable rate technology. Walburg *et al.* (1982) found that when plants are N deficient, spectral reflectance in the near-infrared (NIR) portion of the spectrum decreases, while reflectance in the red increases. Infrared (IR)/red ratio, of reflectance, increases with increased N fertilization and N content in plant tissue (Kleman and Fagerlund, 1987). Nitrogen deficiencies are linked to decreased amounts of leaf chlorophyll and less light absorption. This results in a greater reflectance measurement (Blackmer *et al.*, 1994). Red has been measured at $0.671 \pm 0.006 \mu\text{m}$, NIR at $0.780 \pm 0.006 \mu\text{m}$ (Stone *et al.*, 1995), and green spectral radiance at $0.550 \pm 0.006 \mu\text{m}$ (Thomas and Oerther,

1972; Blackmer, 1994). Stone *et al.* (1996) found plant N spectral index (PNSI) was highly correlated with estimates of N uptake in wheat forage at all stages of growth. The index is calculated as the absolute value of $(NIR + red)/(NIR - red)$ and is the inverse of the normalized difference vegetative index (NDVI). The highest PNSI reading should correspond to the lowest rate of N applied (0 kg ha^{-1}) preplant.

Spectral readings of the wheat forage are taken using photodiode based sensors fitted with interference filters and interfaced to an embedded microcontroller. The readings are used to apply fertilizer just moments after the readings have been obtained based on a predetermined calibration curve (Stone *et al.*, 1995).

Objective

The objective of this study was to evaluate the appropriateness of a variable applied N rate based on a linear calibration curve of NDVI and forage N uptake (biomass x N concentration) versus a fixed topdress N application in field-scale trials.

Literature Review

Remote sensing of radiant energy in the visible and near infrared region may be an input or basis for variable rate technology (VRT) to apply only the amount of fertilizer needed by the plant. VRT is a potentially useful tool for U.S. farmers because it allows them to conserve fertilizer, while maximizing yield potential. With concern about groundwater contamination and the depressed farm economy, the development of VRT for fertilizer application is clearly desirable. Fixed rate fertilizer application does not allow optimum efficiency or profitability (Sawyer, 1994).

Spectral radiance is measured in terms of wavelength. Chlorophyll absorbs light at wavelengths of 0.450 to 0.670 μm . Blue light is measured at about 0.450 μm , while red light is approximately 0.675 μm . Finally, green light is found at 0.550 μm . Approximately 75 to 90% of the light absorbed by green leaves is blue and red. Absorption is approximately 20% for green light (Thomas and Oerther, 1972). Plant leaves reflect wavelengths between 0.700 and 1.300 μm from their internal structure. Above 1.300 μm , energy incident upon vegetation is absorbed or reflected with marginal transmittance of energy (Thomas and Oerther, 1972).

Factors affecting soil reflectance include: soil moisture content, texture, surface roughness, ferric oxide content and organic matter. Sandy soils have a low moisture content and high reflectance. Water affects reflectance between 1.3 and 2.5 μm (Kleman and Fagerlund, 1987). In plants, leaf thickness impacts

light absorption and reflectance (Woolley, 1971). Lillesaester (1982) demonstrated that reflectance on one or two layers of leaves, for wavelengths beyond 0.700 μm , was affected by background (soil) reflectance.

Blackmer *et al.* (1994) found the optimum wavelength for separation of nitrogen (N) treatment differences to be 0.550 μm . They measured chlorophyll activity at 0.650 μm . Moreover, their results indicated chlorophyll activity was proportional to leaf N concentration. Nitrogen deficiencies led to decreased amounts of leaf chlorophyll, which resulted in less chlorophyll light absorption. Therefore, the greatest reflectance occurred with the lowest N rate (Blackmer *et al.*, 1994). In support of this finding, Takebe *et al.* (1990) found N content in the upper leaves relates closely to the intensity values of the canopy green color. When plants are N deficient, reflectance in the red increases, while the near infrared decreases (Thomas and Oerther, 1972). Under nitrogen deficiency, reflectance in sweet pepper (*Capsicum annuum* L.) leaves increases in the visible (0.4 to 0.7 μm) and near infrared (0.7 to 1.4 μm) while decreasing in the middle infrared (1.4 to 2.5 μm) (Walburg *et al.*, 1982). Kleman and Fagerlund, (1987) report that the IR/red ratio increases with increased fertilization in barley. Their findings also show a strong correlation between IR/red ratio and biomass, but the function changes with growth stage. Therefore, it is important to take all of the samples at the same growth stage (Kleman and Fagerlund, 1987).

Water content and internal leaf structure impacts near-infrared reflectance. Intercellular air spaces increased when N deficiency became more severe, as evidenced by increased reflectance (Thomas and Oerther, 1972).

Research by Frederick and Camberato (1995) showed that high rates of spring-applied N increased the severity of drought stress on non-irrigated winter wheat. Furthermore, Karrou and Maranville (1994) noted that severe water stress masked the effect of N supply. Foth (1990) stated that under low nutrient supply, plants would use more water. In addition, increased plant growth results in increased water use efficiency. In humid regions, fertilized crops were more drought tolerant. However, in subhumid regions, if fertilizer caused vigorous growth early in the season, and then the crops were subjected to drought, yields decreased (Foth, 1990).

Vegetation indices are used to enhance vegetation signals from remote sensing instruments. Walburg *et al.* (1982) found the IR/red reflectance ratio enhanced treatment differences in canopy reflectance and reduced reflectance variability from extraneous factors. Kleman and Fagerlund (1987) reported the IR/red ratio indicated the amount of biomass in barley on each measurement date until approximately 20 July. The average of the IR/red ratio during the middle of the season was highly correlated ($r^2=0.97$) to grain yield two months later (Kleman and Fagerlund, 1987).

Leaf area index (LAI) is an important consideration when measuring canopy reflectance. A lower LAI results in decreased canopy reflectance in the near infrared and increased in the red, without any alteration of the reflectance properties of individual leaves (Colwell, 1974). Bunnik (1978) showed that the NIR/red reflectance ratio was highly correlated to variation in LAI of vegetative

canopies, while being relatively insensitive to variations in soil background reflectance.

According to Stone *et al.* (1995), a strong relationship between PNSI and total N uptake exists. Stone *et al.* (1995) found the plant-nitrogen-spectral index (PNSI) was highly correlated with estimates of N uptake in wheat forage at all stages of growth. Work done at Oklahoma State University indicates that forage N uptake is better correlated to grain yield than total N in the forage or forage yield; however, work by Roth *et al.* (1989) showed plant N concentration to be a better predictor of grain yield.

Materials and Methods

Experimental sites were established in Oklahoma, in the fall of 1995 at Altus and Hennessey (1996 crop), and in fall of 1996 at Hennessey and Tipton (1997 crop). Soil types and initial soil test results are reported in Table 1. The soil test data reported is from two and three composite surface soil samples (0-15 cm) of the field in which each experiment was located. The experimental design was a completely randomized block. Four treatments were replicated five times at Altus, Hennessey (1996-97), and Tipton, and 10 times at Hennessey (1995-96). The large numbers of replications were used to test an experimental applicator and to increase our database. Main plots included a check (no N fertilization), a variable N rate (based on NDVI), and two fixed topdress rates of 45 and 90 kg N ha⁻¹ in 1995-96 and 56 and 112 kg N ha⁻¹ in 1996-97. Each main

plot was split into 10 subplots for variable fertilizer application during topdressing and grain harvest in the variable plots. Main plot sizes at both locations were 3.048 m x 15.24 m in 1995-96 and 3.048 m x 21.336 m in 1996-97. Plot size was altered for the 1996-97 crop year to facilitate harvesting. Winter wheat (*Triticum aestivum* L.), 'Tonkawa' variety, was planted at a rate of 79 kg ha⁻¹ in 19 cm row spacing at Hennessey and 27 cm row spacing at Altus and Tipton.

UAN was applied on 26 March 1996 at Altus, 2 April 1996 at Hennessey, 4 February 1997 at Tipton, and 13 February 1997 at Hennessey to the fixed rate and variable rate plots. Tables 2 through 5 report the fertilizer rates that were applied to each subplot in the variable treatments. Wheat at all locations was at either Feekes growth stages 6 or 7 in 1996 and at Feekes growth stage 5 in 1997 when topdress N was applied. Fixed N rates of 45, 56, 90 and 112 kg N ha⁻¹ were applied using a conventional sprayer to support 1344, 1680, 2688, and 3360 kg ha⁻¹ yield goals, (not considering a soil test). Variable rates were applied using portable backpack sprayers, to 3.0 m x 1.5 m plots in 1996 and 3.0 m x 2.1 m plots in 1997. Check plots did not receive any N fertilizer. The fixed rate (90 kg N ha⁻¹ in 1996 and 112 kg N ha⁻¹ in 1997) applications were based on the rate a producer was likely to use for his yield goal.

Spectral readings of the wheat forage in the variable rate plots (1996) and all of the plots (1997) were taken using photodiode based sensors fitted with interference filters and interfaced to an embedded microcontroller (Stone *et al.* 1995). A linear calibration relating spectral readings to forage characteristics

was prepared utilizing data taken by the same sensor from that same site either on that day or two days before. The highest NDVI value corresponded with the highest forage total N uptake. Thus, that plot received no topdress N. Conversely, the lowest NDVI value corresponded with the lowest forage total N uptake, and thus received the a topdress N application of 90 kg N ha⁻¹ in 1996 and 112 kg N ha⁻¹ in 1997. A linear curve was drawn through the NDVI values, and fertilizer was applied accordingly. Outliers at the top and bottom of the NDVI values were discarded when present.

In late May and early June of both years, grain was harvested with a Massey Ferguson 8XP research combine. Harvested area in 1996 was 1.5 m x 2.0 m and 3.0 m x 2.0 m in 1997. Grain yield and grain moisture were recorded using a Harvest Master data system. Grain was ground to pass a 140 mesh screen and analyzed for total N on a Carlo-Erba NA 1500 dry combustion analyzer. Amount of N available for plant uptake was calculated by adding ppm NH₄ and ppm NO₃ and then multiplying by 2.24 to obtain kg N ha⁻¹. Analysis of variance using subplots as sampling error was performed (SAS Institute, 1985). Contrasts were also evaluated for the linear effect of N rate, VRN versus the check, VRN versus the one-half fixed rate, and VRN versus the fixed rate.

Results

Response to applied N fertilizer was variable in the both years of the experiment. Hence, the detailing of results that follows was separated by year

and location. Analysis of variance for each site is presented in Tables 6 through 9.

Altus, 1996

A significant linear response to applied N was observed for grain yield, total N in the grain, and grain N uptake (Table 6). Grain yield and grain N uptake decreased with increasing applied N, while total N concentration in the grain increased. The extremely low grain yields were attributed to severe freeze damage and drought encountered at this site. Sufficient soil $\text{NH}_4\text{-N}$ plus $\text{NO}_3\text{-N}$ were present to produce 934 kg ha^{-1} grain without added fertilization. Regression analysis indicated there was no correlation at this site between NDVI readings taken from the variable rate plots on 2 March 1996 at Feekes growth stage 6 and grain yield, total N in the grain, or grain N uptake. No differences were found between the variable rate N (VRN) (48 kg N ha^{-1}) and either of the fixed N rates. Yield response when N was variably applied was independent of NDVI. This affirms the contrasts that under drought conditions N level did not affect yield (Table 6).

Hennessey, 1996

A slight trend for applied N fertilizer to decrease grain yield was found at Hennessey (Table 7). The lack of differences in yield restricts the comparison of N treatments, especially compared to VRN, because N savings would be inconsequential. No differences were found between the variable rate N (VRN)

(43 kg N ha⁻¹) and either of the fixed N rates and the check. In the VRN treatment, yields were considerably reduced at higher topdress N rates.

Tipton, 1997

At Tipton, the main effect of N fertilizer rate was significant for grain yield, total N in the grain, and grain N uptake, with grain yield increasing from 539 to 797 kg ha⁻¹ and total N in the grain from 28.2 to 29.7 g kg⁻¹ from the check to fixed N rate plots, respectively (Table 8). Normal yields in this county were 1206 kg ha⁻¹ higher. The large yield reduction was the result of freeze damage on April 11, 12 and 13. Actual yields ranged from 797 kg ha⁻¹ for the 112 kg N ha⁻¹ to 539 kg ha⁻¹ for the check. The variable rate N application (VRN) (averaging 45 kg ha⁻¹) had lower grain yield (649 kg ha⁻¹) and grain N uptake (19 kg N ha⁻¹) compared to the fixed rate of 112 kg N ha⁻¹ rate (797 kg ha⁻¹ and 24 kg N ha⁻¹). Total N in the grain was lower in the VRN plot (28.9 g kg⁻¹) compared to the 56 kg N ha⁻¹ (29.5 g kg⁻¹).

Hennessey, 1997

Response of grain yield and grain N uptake to N fertilizer was linear (Table 9). The variable rate N application (46 kg ha⁻¹) had lower grain yield, total N in the grain and grain N uptake compared to the fixed rate of 112 kg N ha⁻¹. Figure 2 shows grain yield, total N in the grain and grain N uptake in the subplots within check plots (no N applied) plotted against NDVI for the check plot. Grain yield and grain N uptake increased with increasing NDVI readings taken at an early stage of growth. This implies that at Feekes growth stage 5,

NDVI was able to detect some differences in forage N uptake between plots and which could be used to predict yield as has been reported by Stone *et al.* (1995) and Tucker *et al.* (1980).

Discussion

Altus, 1996

The Altus 1995-96 growing season, September through May, was very dry, with total rainfall only reaching 264 mm. Normal rainfall for this period is 449 mm. Thus, it was not startling that the effect of N fertilizer application was not significant since response was controlled by moisture and not nutrient limited. Interestingly, the 90 kg N ha⁻¹ rate reduced yield, confirming the conclusions of Frederick and Camberato (1995) and Karrou and Maranville (1994) that in drought conditions application of N can reduce yield.

Even though wheat was at Feekes growth stage 6 when the readings were taken, the wheat canopy was at low coverage. Unpublished data by Lees *et al.* (in preparation) suggests NDVI is not a good predictor of N uptake until percent vegetative coverage reaches or exceeds 50%. The very low range in NDVI from 0.18 to 0.28 is usually associated with bare soil. However, the readings for this site were taken under overcast skies which shift the values lower and reduce sensitivity. In addition, vegetation was very sparse due to drought. The drier than normal year, freeze injury that occurred in January, and subsequent lack of vigorous growth contributed to the lack of correlation between NDVI and grain yield, total N in the grain, and grain N uptake. The

biggest reason for lack of NDVI response was extremely low yield. Sufficient N was present in the soil to support a yield of 940 kg ha⁻¹ without additional fertilizer N. Average yield for the VRN treatment was 605 kg ha⁻¹ which equaled the check yield. Under this condition, no response should be expected. Hence, due to the environmental factors present in this portion of the study, no inferences on the justification of sensor-based variable rate technology should be made from data at this site.

Hennessey, 1996

In addition to low rainfall at Hennessey (261 mm Sept 1995 through May 1996), enough soil inorganic N was present, without the addition of fertilizer, to produce 2600 kg ha⁻¹ wheat (Table 7). Annual rainfall for this area averaged 543 mm. Average yields for each treatment were less than 2020 kg ha⁻¹. Thus, we expected limited, and perhaps negative, response to applied fertilizer N. Since fertilizer was applied relatively late in the growing season under drought conditions (end of Feekes growth stage 6), limited uptake of applied fertilizer N occurred. This was compounded by high inherent soil N levels. Under these conditions the topdress fertilizer application reduced yield about 100 kg ha⁻¹. This trend was observed in the VRN plots where yield tended to decrease with increasing N application.

Tipton, 1997

In contrast to the previous growing season, in 1996-97, total rainfall for Tipton was 542 mm (Sept. 1996 through May 1997) and for Hennessey was 599

mm (Sept. 1996 through Apr. 1997). Normal rainfall for the two areas was 520 mm and 543 mm, respectively. Initial soil test results from Tipton showed 29 kg N ha⁻¹ available for plant uptake, or enough to produce 874 kg of wheat grain. As there was adequate moisture supply, a yield response to applied fertilizer N was expected. The April freeze drastically lowered the yields for this location from an average historic yield of 2000 kg ha⁻¹ to 665 kg ha⁻¹. Even though the yield goal for the fixed rate plots was 3360 kg ha⁻¹, the 112 kg N ha⁻¹ treatment only averaged 850 kg grain ha⁻¹. The fact that grain yield and grain N uptake decreased with increasing NDVI values, suggests that the subplots with the highest NDVI readings needed some amount of fertilizer, too. However, with yields this low, it is difficult to reliably evaluate treatments or draw conclusions.

Because of this we think the high end of the variable N rate treatment was not high enough since yields and N uptake were lower. Also, the improved yields (112 kg N ha⁻¹ vs. VRN) could suggest that the variability in NDVI detected, and which was the basis for the linear NDVI-N rate fertilizer recommendation may not have been appropriate. The variability in NDVI detected may not have been due to deficiencies of N, the linear calibration curve may have underestimated N need. Previous work by Solie *et al.* (1996) suggests an exponential curve may be a better fit. See Figure 1 for NDVI versus grain yield, total N in the grain, and grain N uptake in the check plot. Also, it was important to find that the NDVI range was narrow, 0.5 to 0.65, indicating that there was uniform vegetation and uniform N concentration.

Hennessey, 1997

Hennessey had less freeze damage than Tipton. Consequently, forage N uptake was related to yield parameters for this particular year at Hennessey. Figure 2 shows NDVI versus grain yield, total N in the grain, and grain N uptake for the check plot at Hennessey. Growth was more vigorous at this site than the others, having achieved 50% vegetative coverage by late January. Although the NDVI readings were uncalibrated, the values for this location and year were higher than any of the others.

Three other factors may be responsible for the lack of correlation between NDVI and yield parameters. The N rates applied may not have been appropriate for the site. It is possible that the high end of the linear curve should have received 20 kg N ha⁻¹ instead of 0, or that the plots needing the highest fertilizer application occurred in the middle of the curve. Furthermore, the slope of the actual curve relating NDVI to forage N uptake may not have been linear. In addition, the variability detected with NDVI may have been due to factors other than N.

Conclusion

The wide scatter in the data points over a narrow NDVI range in the Hennessey (1996-1997) data implies NDVI was not sensitive to forage N uptake, probably due to the fact that it could not detect canopy depth; or there was an intervening cause and N was adequate. This poses a question: should we be attempting to fertilize based on growth stage, percent coverage, or at a time

where plant coverage is at 50% or greater, but when the plant has not achieved much height? Perhaps the solution is an ultrasonic device to estimate plant height and integrate that with NDVI. Also, since NDVI was apparently unable to sense forage N uptake at Feekes growth stage 5, when high levels of biomass were present, the need for an independent in-field estimation of forage N uptake becomes apparent. This might involve a quick plant tissue test for N content and weighing forage clippings from a specific area. Sensor readings of the area would be taken beforehand and a calibration curve developed. Ideally, technology would advance to the point where in field calibration was no longer necessary. If the resolution on global satellite positioning systems becomes fine enough, it might be used for the experimental applicator to more accurately match NDVI readings with grain yield.

An indication of N uptake (less than or greater than $x \text{ kg N ha}^{-1}$) at x stage of growth is needed prior to establishing the linear N-rate curve. NDVI values where N uptake is known to be greater than $x \text{ kg N ha}^{-1}$ at x stage of growth would not be fertilized. Similarly, the high end may all receive 20 kg N ha^{-1} depending on yield potential. Work by Roth *et al.* (1989) showed whole plant N concentrations necessary to produce 90% of maximum yields or critical levels, were 39.0, 35.0, and 26.5 g N kg^{-1} for Feekes growth stages 4, 5, and 6, respectively.

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Table 1. Initial surface (0-15cm) soil test characteristics and soil classification at Altus, Hennessey, and Tipton, OK.

Location	pH	NH ₄ -N	NO ₃ -N	P	K	Org. C	Total N
		-----mg kg ⁻¹ -----			-----g kg ⁻¹ -----		
Altus	7.9	7.9	6.0	22	466	10.4	0.8
Classification: Tillman-Hollister clay loam (fine, mixed thermic Pachic Paleustoll)							
Hennessey, 1995	5.8	18.4	21.1	142	674	12.3	1.0
Hennessey, 1996	5.3	9.1	16.0	100	498	10.8	1.0
Classification: Shellabarger sandy loam (fine-loamy mixed thermic Udic Argiustoll)							
Tipton	7.4	5.1	7.9	30	465	8.0	0.8
Classification: Tillman-Hollister clay loam (fine, mixed thermic Pachic Arguistoll)							

pH - 1:1 soil:deionized water; NH₄-N and NO₃-N 2M KCl extract, K and P - Mehlich-3 extraction, Organic Carbon and Total N - dry combustion.

Table 2. Nitrogen rates applied to variable-rate subplots, Altus, OK, 1996.

Distance m	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
	-----Nitrogen Rate, kg ha ⁻¹ -----				
1.52	56	30	77	81	30
3.05	60	34	69	69	11
4.57	54	29	82	64	0
6.10	53	20	74	79	63
7.62	90	39	44	20	73
9.14	61	25	54	58	51
10.67	51	17	60	80	24
12.19	52	5	64	30	44
13.72	63	33	60	35	28
15.24	55	35	45	24	22
Average variable rate applied:		48 kg N ha ⁻¹			

Table 3. Nitrogen rates applied to variable-rate subplots, Hennessey, OK, 1996.

Distance m	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
	Nitrogen Rate kg ha ⁻¹									
1.52	21	83	15	74	29	43	37	46	44	88
3.05	20	13	27	27	27	65	90	66	42	40
4.57	27	17	13	9	57	52	73	43	90	9
6.10	14	20	13	33	66	63	55	39	60	6
7.62	17	38	19	27	25	65	56	51	46	37
9.14	33	65	30	25	26	36	66	56	77	50
10.67	28	40	38	29	29	55	88	60	53	38
12.19	44	31	51	32	35	37	62	88	15	0
13.72	47	46	61	35	32	41	88	69	37	8
15.24	48	30	57	42	35	50	65	60	90	2
Average variable rate applied: 43 kg N ha ⁻¹										

Table 4. Nitrogen rates applied to variable-rate subplots, Tipton, OK, 1997.

Distance m	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
	-----Nitrogen Rate, kg ha ⁻¹ -----				
2.13	72	42	60	58	38
4.27	77	71	66	32	73
6.40	45	89	80	31	45
8.53	42	62	71	8	46
10.67	58	66	60	17	52
12.80	60	35	25	15	43
14.94	70	39	19	22	63
17.07	23	55	8	27	67
19.20	18	19	16	26	29
Average variable rate applied:		45 kg N ha ⁻¹			

Table 5. Nitrogen rates applied to variable-rate subplots, Hennessey, OK, 1997.

Distance m	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
	-----Nitrogen Rate, kg ha ⁻¹ -----				
2.13	112	112	52	74	17
4.27	86	45	46	38	39
6.40	64	81	12	9	47
8.53	112	72	47	35	30
10.67	62	60	34	31	14
12.80	68	49	35	17	44
14.94	79	71	35	8	10
17.07	42	61	27	8	40
19.20	49	39	32	0	33
21.34	56	57	31	16	29
Average variable rate applied:		46 kg N ha ⁻¹			

Table 6. Analysis of variance and single-degree of freedom contrasts for Altus, OK, 1995-96.

Source of variation	df	Grain yield, kg ha ⁻¹	Grain total N, g kg ⁻¹	Grain N uptake, kg N ha ⁻¹
		-----Mean Squares-----		
Rep	4	315503.52	64.29	149.90
Nrate		45114.88	12.23	19.19
3				
<u>Contrasts</u>				
N rate linear		92890.89***	30.71***	44.14**
VRN vs 0		81.29	1.59	0.001
VRN vs 45		1344.02	0.44	1.45
VRN vs 90		98468.03***	17.05**	41.37**
		-----Treatment Means-----		
<u>Nitrogen rate</u>				
(kg ha ⁻¹)				
0		383 ± 131	27.6 ± 2.2	10 ± 3
45		380 ± 123	27.7 ± 2.0	10 ± 3
90		323 ± 101	28.6 ± 2.0	9 ± 3
VRN (48 kg ha ⁻¹)		389 ± 115	27.8 ± 1.6	11 ± 3
CV		40	9.9	33
SED		30	0.6	0.7

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

VRN = variable rate N applied (average = 48, min = 0, max = 90 kg ha⁻¹)

CV = coefficient of variation

SED = standard error of the difference between two equally replicated treatment means.

Table 7. Analysis of variance and single-degree of freedom contrasts for Hennessey, OK, 1995-96, backpack sprayer method.

Source of variation	df	Grain yield, kg ha ⁻¹	Grain total N, g kg ⁻¹	Grain N uptake, kg N ha ⁻¹
-----Mean Squares-----				
Rep	4	1976861.59	14.44	1940.80
Nrate	3	189232.65	6.75	146.75
<u>Contrasts</u>				
N rate linear		499543.10*	6.6	326.49
VRN vs 0		134844.96	2.39	252.31
VRN vs 45		40929.19	10.85*	1.52
VRN vs 90		113984.61	16.90**	4.75
-----Treatment Means-----				
<u>Nitrogen rate</u> (kg ha ⁻¹)				
0		2072 ± 421	32.9 ± 1.5	68 ± 14
45		1991 ± 430	33.2 ± 1.8	66 ± 14
90		1972 ± 381	33.3 ± 1.7	66 ± 13
VRN (43 kg ha ⁻¹)		2017 ± 472	32.7 ± 1.2	66 ± 15
CV		27	7.3	27
SED		78	0.3	2.5

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

VRN = variable rate N applied (average = 43, min = 0, max = 90 kg ha⁻¹)

CV = coefficient of variation

SED = standard error of the difference between two equally replicated treatment means.

Table 8. Analysis of variance and single-degree of freedom contrasts for Tipton, OK, 1996-97.

Source of variation	df	Grain yield, kg ha ⁻¹	Grain total N, g kg ⁻¹	Grain N uptake, kg N ha ⁻¹
-----Mean Squares-----				
Rep	4	13684.88	2.83	9.90
Nrate		559307.54**	22.36***	601.10***
3				
<u>Contrasts</u>				
N rate linear		1658897.84***	57.87***	1773.96***
VRN vs 0		301625.56**	12.75**	319.79**
VRN vs 56		17351.59	6.89*	28.88
VRN vs 112		545792.34***	16.30**	587.37***
-----Treatment Means-----				
<u>Nitrogen rate</u>				
(kg ha ⁻¹)				
0		539 ± 187	28.2 ± 1.4	15 ± 5
56		675 ± 179	29.5 ± 1.2	20 ± 5
112		797 ± 178	29.7 ± 1.1	24 ± 5
VRN (45 kg ha ⁻¹)		649 ± 219	28.9 ± 1.2	19 ± 6
CV		36	3.0	36
SED		47.3	0.2	1.4

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

VRN = variable rate N applied (average = 45, min = 0, max = 112 kg ha⁻¹)

CV = coefficient of variation

SED = standard error of the difference between two equally replicated treatment means.

Table 9. Analysis of variance and single-degree of freedom contrasts for Hennessey, 1996-97.

Source of variation	df	Grain yield, kg ha ⁻¹	Grain total N, g kg ⁻¹	Grain N uptake, kg N ha ⁻¹
-----Mean Squares-----				
Rep	4	2344786.76	109.96	1162.33
Nrate	3	767339.46	12.32	485.33

<u>Contrasts</u>				
N rate linear		1725990.56**	6.79	1209.96**
VRN vs 0		1690.91	6.28	17.96
VRN vs 56		37540.95	0.02	31.06
VRN vs 112		1619635.38**	26.14**	933.10*

-----Treatment Means-----				
<u>Nitrogen rate</u>				
(kg ha ⁻¹)				
0		1391 ± 483	31.1 ± 2.7	43 ± 14
56		1438 ± 434	31.6 ± 2.2	45 ± 12
112		1654 ± 502	30.5 ± 2.5	50 ± 13
VRN (46 kg ha ⁻¹)		1399 ± 461	31.6 ± 2.1	44 ± 13
CV		47.09	10.77	41.62
SED		138.5	0.7	3.8

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

VRN = variable rate N applied (average = 46, min = 0, max = 112 kg ha⁻¹)

CV = coefficient of variation

SED = standard error of the difference between two equally replicated treatment means.

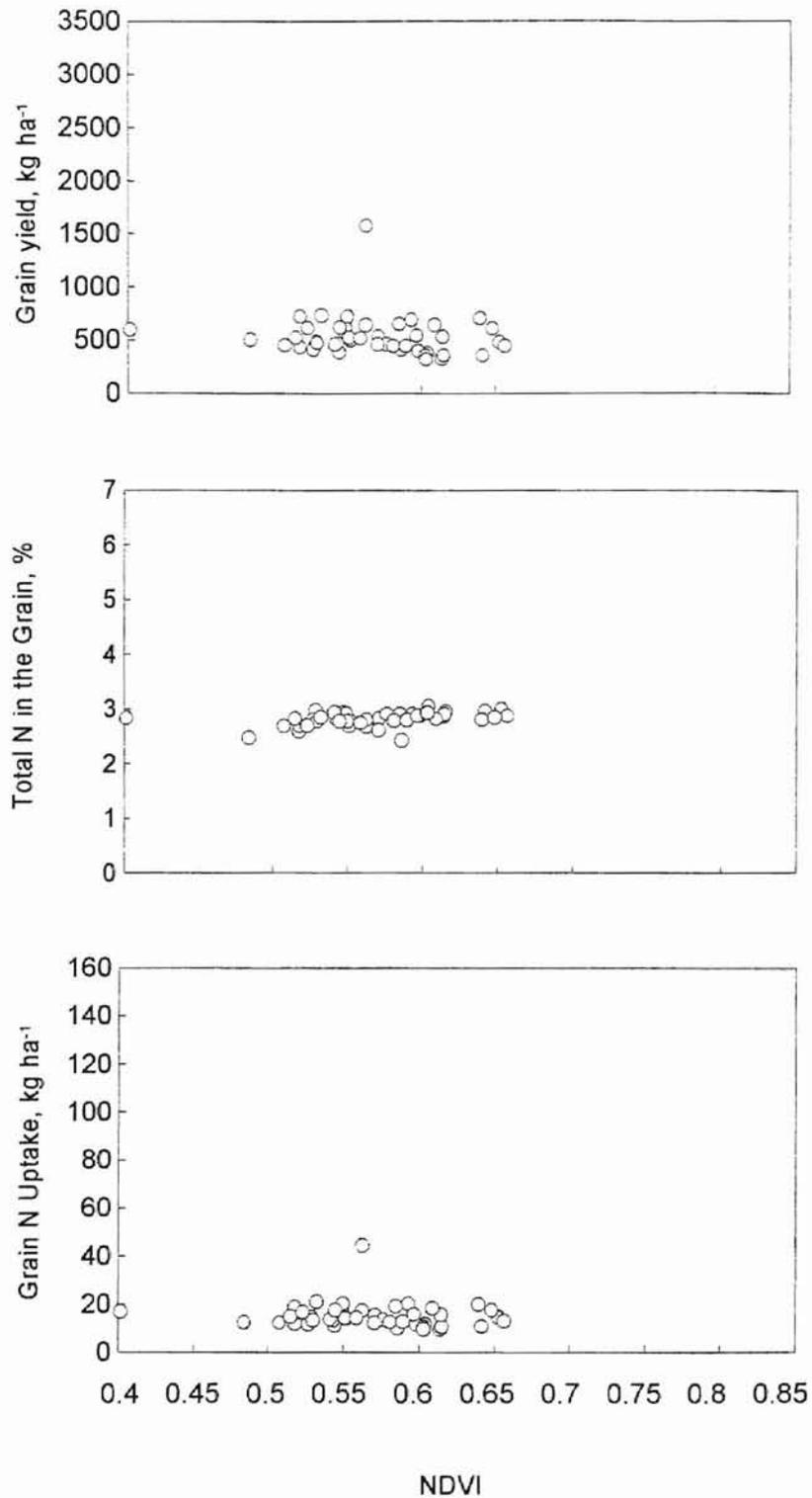


Figure 1. Correlation of grain yield, total N in the grain, and grain N uptake with NDVI (4 Feb 1997, Feekes growth stage 5) on check plots, Tipton, OK, 1997

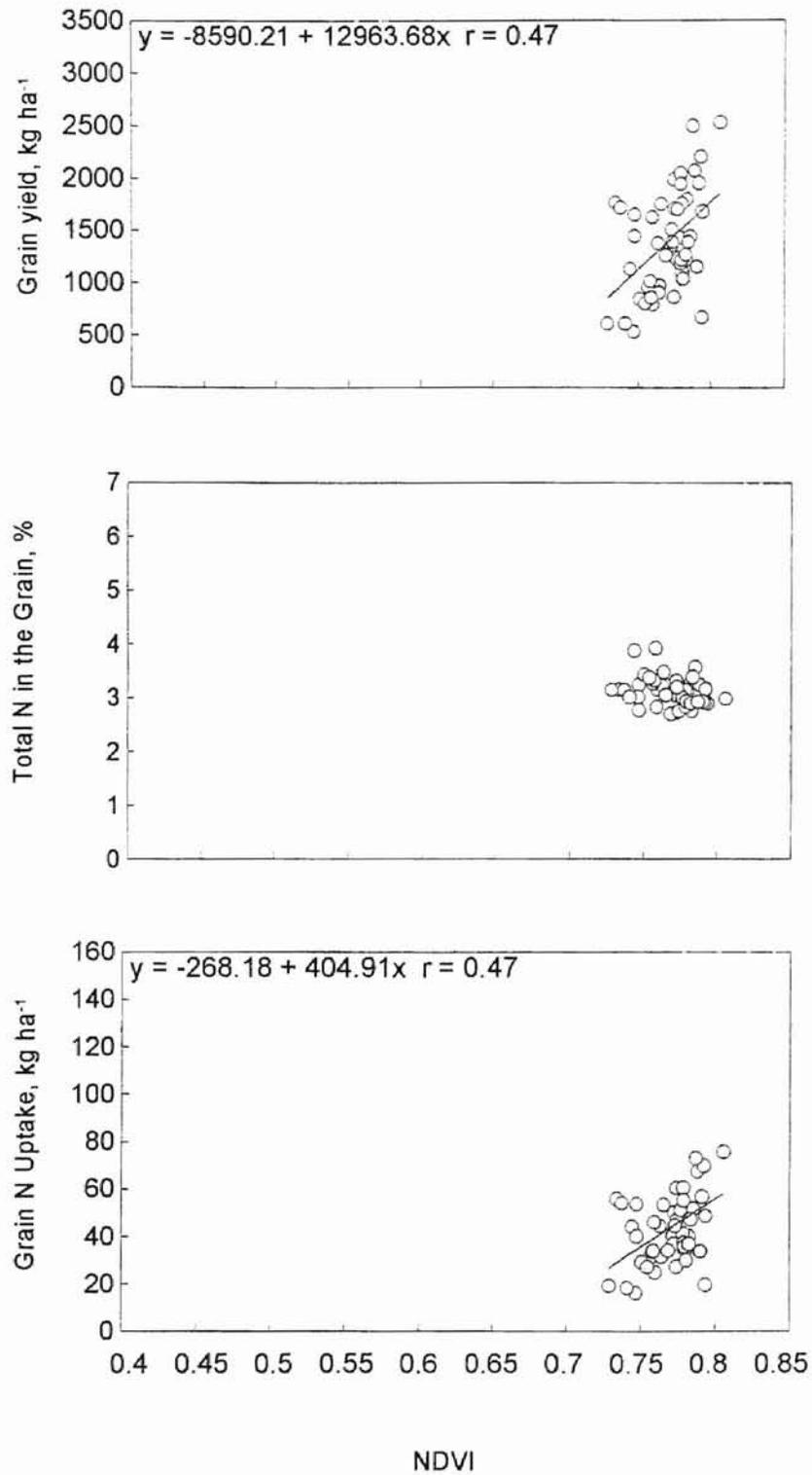


Figure 2. Correlation of grain yield, total N in the grain, and grain N uptake with NDVI (11 Feb 1997, Feekes growth stage 5) on check plots, Hennessey, OK, 1997

II. Estimating Gaseous Nitrogen Losses From *Triticum aestivum* L. Using ^{15}N

Introduction

Nitrogen (N) fertilizer is generally the most costly input for winter wheat (*Triticum aestivum* L.) production. Therefore, it is important to maximize fertilizer use efficiency and minimize nitrogen losses to the environment. These losses occur from the soil plant system via denitrification, volatilization (both from the plant and the soil), and leaching. By understanding N movement in the soil, we can characterize how these losses occur and seek to increase nitrogen use efficiency (NUE). One of the mechanisms responsible for decreased NUE is plant N loss.

Nitrogen movement through the soil over time is difficult to measure without the use of ^{15}N . By enriching fertilizer with ^{15}N , researchers can quantify the amount of N present in soil and plant fractions due to the fertilizer. The percent natural abundance of ^{15}N is 0.3663, while that of ^{14}N is 99.634% (Schepers *et al.*, 1989, Vose, 1980 and Hauck and Bremner, 1976). Therefore, an enriched ^{15}N fertilizer material contains more than 0.3663% ^{15}N atom excess (Vose, 1980).

Work by Raun and Johnson (1995) indicates that the soil-plant system can buffer against soil profile inorganic N accumulation when N rates exceed that required for maximum yield. Plant N loss is one of the buffering mechanisms (Raun and Johnson, 1995). Francis *et al.* (1993) found plant N loss

was responsible for 52-73% of the undetermined N loss. In addition, Harper *et al.* (1987) found 21% of applied fertilizer N was lost as volatile ammonia during senescence in wheat.

Nitrogen use efficiency (NUE) is equal to grain weight divided by the nitrogen supply (Moll *et al.*, 1982). Moll *et al.* (1982) found significant differences in NUE between all N rates. Lower NUE was found at higher N rates. Wuest and Cassman (1992) showed 30-55% recovery of N applied at planting and 55-80% recovery of N applied at anthesis for irrigated hard red spring wheat. Furthermore, post-anthesis N uptake was not increased by greater preplant fertilizer N levels (Wuest and Cassman, 1992).

Objectives

The objectives of this experiment were to 1.) To determine fertilizer N recovery in winter wheat when produced for forage and grain, 2.) To quantify potential plant N losses from flowering to maturity in winter wheat, and 3.) To estimate residual fertilizer effects.

Literature Review

Nitrogen 15 (^{15}N) is often used to label fertilizers because it allows the researcher to trace fertilizer N movement in the soil. It was applied here to further investigate N buffering mechanisms and nitrogen use efficiency (NUE). First, one must understand the basic soil processes occurring and ^{15}N 's application.

¹⁵N labeled fertilizers

Research has been conducted on ¹⁵N techniques and analysis methods. This includes the use of microplots, comparison of N recovery methods, and analysis with a mass spectrometer.

Due to the high cost of ¹⁵N fertilizer, microplots are used for ¹⁵N experiments. ¹⁵N research is valuable because it allows the researcher to trace specific N sources' movement and behavior in the soil. Fertilizer applied to microplots is confined to the plot by using a sheet metal cylinder or square inserted into the soil surface (Carter *et al.*, 1967; Olson *et al.*, 1979; Kowalenko, 1989; Harris *et al.*, 1994). This method enables researchers to confine lateral fertilizer movement (Carter *et al.*, 1967).

There are two methods for determining nitrogen recovery, the difference method and the isotope-ratio analysis method. Walters and Malzer (1990) found that when they used the difference method, fertilizer N leaching losses in corn were three times greater than under isotope-ratio analysis. They attributed this difference to isotope dilution with indigenous soil N due to microbial activity. Varvel and Peterson (1990) found significantly different nitrogen recovery percentages between the two methods. Despite the difficulties encountered with the use of either method, both remain valuable tools.

Hauck and Bremner (1976) outline the assumptions under both methods. The difference method assumes that mineralization, immobilization, and other N transformations are the same for fertilized and unfertilized soils. The isotope-

ratio analysis method assumes that isotope compositions in tracers are static, living organisms cannot differentiate between isotopes of the same element, and isotope dilution does not occur in biochemical processes. N loss during drying and preparation of wet soil samples could result in error (Hauck and Bremner, 1976). They state the only significant errors encountered with mass spectrometer analysis are N₂, N₂O, CO₂, and volatile amine contamination during sample preparation.

Westerman and Kurtz (1972) noted past methods for measuring residual effects were normal difference and/or linear regression. However, these only measure total N and are not capable of identifying which atoms come from native soil N and those coming from applications of fertilizer N. Therefore, ¹⁵N techniques are necessary to effectively characterize soil N.

Isotopic nitrogen samples may be analyzed using Carlo Erba model NA 1500 automatic N analyzer interfaced to a mass spectrometer (Schepers *et al.*, 1989). Schepers *et al.* (1989) did not find a memory effect in between samples. The memory effect is associated with the formation of nitric acid and its adsorption to the inner surfaces of the dry combustion unit and mass spectrometer. (Schepers *et al.*, 1989). Calculations can be made based on the equation from Vose (1980).

Recovery

Westerman *et al.* (1972) conducted experiments on *Sorghum sudanense* over two years to study N recoveries from urea and oxamide fertilizers. In the

first year, with urea fertilizer, 51% of the applied N was recovered in the crop and 28% of applied fertilizer N remained in the top 25 cm of the soil profile; while with oxamide fertilizer, 52% was recovered in the crop and 31% in the soil. In the following year, when planting and fertilizer (unlabeled) application were delayed due to weather conditions, recoveries for the crop were 93% for urea and 99% for oxamide. Soil recoveries in the second year were not measured. Toward the end of the season, little or no response in yield was found due to fertilizer use. Furthermore, the amount of fertilizer N remaining in the soil after cropping, increased significantly as rates of N increased, except at urea rates of 112 to 168 kg N ha⁻¹. Unaccountable N loss was attributed to possible leaching and denitrification.

Carter *et al.* (1967) found total fertilizer recovery, eight weeks into the growing season, to be between 88 and 96%. Greater recovery, and greater plant uptake, was found from ammonium than from nitrate sources. Carter *et al.* (1967) found no significant difference in recovery of the fertilizer N due to the percent of excess ¹⁵N in the fertilizer, size of plot, exposure to natural rainfall, cropping, N source, or the time of application of fertilizer. Thus, ¹⁵N is ideal for use in tracer experiments. However, recovery of excess ¹⁵N varied widely with core sampling (Carter *et al.*, 1967).

Further experimentation by Reddy and Reddy (1993) found that corn used three to six times more soil N than fertilizer N and hence concluded that extensive turnover through immobilization and mineralization was occurring. N losses ranged from 11 to 48% depending on the level of fertilizer application.

After the growing season, they found most of the soil N in the organic fraction. Westerman and Kurtz (1972) found residual N in the soil at the end of the second cropping season of sudangrass to be 22%.

Of the surface applied fertilizer N, 71 to 77% remained in the 0 to 0.1 m soil profile (Olson and Swallow, 1984). Carter *et al.* (1967) also found most of the applied N at the beginning of the growing season was in the top 0 - 15 cm of the soil profile, and 9.6 - 11.5% of that remained in the inorganic state. Also according to Olson and Swallow (1984) fertilizer N in the soil profile (1.8 m) increased each year. At the end of five years, 54% of applied fertilizer N remained in the 50 kg ha⁻¹ treatments and 47% in the 100 kg ha⁻¹ treatments. Olson *et al.* (1979) found 70-75% of the total fertilizer N was in the top 10 cm of the soil profile, while 70-91% was in the top 20 cm.

Other research examined the issue of applied N retention in the soil. Harris *et al.* (1994) stated that 17% of the applied fertilizer N was retained in the soil. Carter *et al.* (1967) found most of the applied N was either taken up into the plant or kept in the top 15 cm of the soil profile. Webster *et al.* (1986) found 22% of the fertilizer applied in the first soil remained in the soil at the end of the growing season; slightly more than half of which was found in the upper 15 cm. Walters and Malzer (1990) found the soil contained 31% applied fertilizer N.

Westerman and Kurtz (1972) looked at residual effects of fertilizer. In the second cropping season after ¹⁵N labeled fertilizer had been applied, 1.5% and 1.7% of total N in the plant tops from the second harvest was from applications the previous year of urea and oxamide, respectively. Amount of residual N

increased in the 0 - 25 cm soil profile with increasing N application rate. With all of the rates averaged, residual N values at the end of the second cropping season for urea and oxamide were 16 and 20%, respectively. Two-thirds of the residual N was in the 0-10 cm layer (Westerman and Kurtz, 1972). Moreover, Reddy and Reddy (1993) found the first 15 cm of the soil profile contained one-half of the residual N.

Various researchers measured other parameters related to the use of ^{15}N in fertilizer studies. Reddy and Reddy (1993) discovered that in the organic N fraction labeled N recovery decreased with increasing depth. Follet *et al.* (1991) applied liquid ^{15}N enriched fertilizer (sprayed) to microplots and granular fertilizer (broadcast) to the area outside of the microplots. They found plant responses to both forms of fertilizer to be the same. Webster *et al.* (1986) discovered the crop contained more labeled N after a period of waterlogging than did the freely drained treatments. Harris *et al.* (1994) found the microbial biomass recovered between 3 and 6% applied fertilizer N. The nonbiomass organic fraction contained 14% of the applied fertilizer N.

Movement of N through the soil profile did not reach beyond the sampling zone during a growing season that received 208 mm of rainfall (Carter *et al.*, 1967). Webster *et al.* (1986) observed little leaching loss (less than 1% in the first year and 1.3 and 3.9% over the 5 and 6 year time periods). In the three winters following the first, a total of 3% of the fertilizer applied in the first year appeared in the leachate for the sandy loam, while only 1% did for the clay soil. Percentage in the leachate from the first year's fertilizer application declined with

each succeeding year, presumably due to dilution and nutrient cycling (Webster *et al.*, 1986). Walters and Malzer (1990) found the leachate collected 1.2 m beneath the surface contained 10% applied fertilizer N. Kowalenko's (1989) research in Canada showed that all of the residual nitrate is leached during the winter months.

Westerman *et al.* (1994) investigated $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ in soil profiles in long-term winter wheat experiments. At fertilizer rates equal to or below that required for maximum yield, $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$ levels did not vary significantly from that of the check. However, at levels greater than that necessary for maximum yield, $\text{NH}_4\text{-N}$ accumulated in the 0-15 cm section of the soil profile, while $\text{NO}_3\text{-N}$ levels accumulated in the subsurface soil profile. Sharpe *et al.* (1988) found plant uptake and immobilization accounted for the rapid decrease in fertilizer N levels in the 0-75 cm soil profile. During the elongation stage of wheat, fertilizer N was immobilized and the plant stopped taking up fertilizer N. At this time, the plant received NH_3 from the atmosphere, until mineralization of fertilizer N began about one month later (Sharpe *et al.*, 1988).

Most of the ^{15}N research done shows that the plant removes between 40 and 58% of the applied fertilizer N in the growing season that the fertilizer was applied in, while the grain removes 27 to 33% (Olson and Swallow, 1984). Research done on clay and sandy loam soils indicated 46 and 58% of applied fertilizer N, respectively, had been taken up by winter wheat shoots by harvest during the first growing season (Webster *et al.*, 1986). Further, studies in corn by Walters and Malzer (1990) showed the plant removed 52% of applied

fertilizer N. Reddy and Reddy (1993), also examining corn, found applied fertilizer N recoveries of 43 to 57% and 17 to 20% in the grain. Webster *et al.* (1986) found after five and six years had passed, total recoveries were 49 and 62%. Harris *et al.* (1994) stated that 40% of applied fertilizer N was removed by the crop. Webster *et al.* (1986) concluded 50 to 60% of applied fertilizer N is recovered by the crop. They also found the shoots contained between one and two percent of the labeled fertilizer in subsequent years.

Shearer and Legg (1975) and Kohl *et al.* (1973) found a consistent decline in the delta ^{15}N with increasing rates of N application. The decline in delta ^{15}N was consistent with increasing contributions of fertilizer N to the plants when the rate of fertilizer N had a lower delta ^{15}N content than the soil N.

Plant N Loss

Work by Raun and Johnson (1995) indicates the soil-plant system can buffer against soil profile nitrate accumulation which would normally occur when N rates exceed that necessary for maximum yield. Using linear-plateau models, it was discovered that maximum yields occurred at N rates less than that required to increase inorganic N accumulation in the soil profile. If the N did not increase yield, and it was not leached, where was it going? Grain N uptake and plant N volatilization increased at N rates in excess of that needed for maximum yield. Increased straw yield, straw N, grain protein and soil organic carbon also occurred.

Plant N loss is one mechanism the plant has for buffering against nitrate accumulation when N rates exceed those necessary for maximum yield (Raun

and Johnson, 1995). Ammonia volatilization can remove excess inorganic N equivalent to 50% of the maximum yield requirement (Johnson and Raun, 1995). Harper *et al.* (1987) reported that plant N concentration reached its maximum point during the vegetative stage in wheat. The plant continued to take up N, although the total plant N declined until maturity. The leaves translocated more N to the grain than did the stem. Daigger *et al.* (1976) found total N losses from 25 to 80 kg ha⁻¹ in winter wheat. In their research, the stem lost 73 to 75% of the N. Stutte *et al.* (1979) discovered that soybeans lost more N during early vegetative growth stages than during flowering and pod fill. Morgan and Parton (1989) found grain fill produced the highest rates of ammonia volatilization in spring wheat, with especially high levels of ammonia volatilization occurring after anthesis and prior to maturity. Work by Altom *et al.* (1996) also demonstrated a constant NUE (60 to 70%) across a range of N rates (56 to 263 kg N ha⁻¹) in a rye-wheat-ryegrass forage production system. Kanampiu (1997) showed decreased NUE with increasing rates of N fertilizer in wheat.

Francis *et al.* (1993) investigated plant N loss from corn. They found 7 - 34 kg ha⁻¹ was lost from aboveground plant biomass. Plant N loss was responsible for 52 -73% of the undetermined loss. Moreover, they found most plant N losses occurred after anthesis, and plant N loss was significantly greater at higher rates of N (Francis *et al.*, 1993). Harper *et al.* (1987) found 21% of applied fertilizer N was lost as volatile ammonia during senescence in wheat. Also, Harper *et al.* (1987) noted approximately 11 % of the potential N available for redistribution from stems and leaves was lost as volatile ammonia. Between

40 and 50% of grain N is taken up from the soil after anthesis (Francis *et al.*, 1993). Plant N loss was maximized at the N fertilization rate where the greatest leaf area occurred. Finally, in corn plants, post-anthesis N losses from aboveground biomass ranged from 10 - 20% of the fertilizer applied (Francis *et al.*, 1993).

Daigger *et al.* (1976) also found total N losses in winter wheat increased with increasing rates of N application. Total N losses in that study were between 25 and 80 kg N ha⁻¹. Work done at Oklahoma State University by Kanampiu (1997) documented plant N losses in winter wheat that ranged from 8 to 26 kg N ha⁻¹. Like the present study showed, he found losses increased with increasing rates of N application. Kanampiu (1995) showed 16.31 kg N ha⁻¹ lost from check plots in low yielding years. Other research in irrigated corn production estimated plant N losses as high as 90 kg N ha⁻¹. Furthermore, plant N losses in corn accounted for 52 to 73% of the unaccounted N using ¹⁵N balance (Francis *et al.*, 1993).

Research by Wuest and Cassman (1992) found post-anthesis N uptake was not increased by greater preplant fertilizer N levels. Contrary to this, Raun and Johnson (1995) found grain N uptake and plant N volatilization increased at N rates in excess of that needed for maximum yield.

Several researchers commented on other factors which may be contributing to N loss, including the type of fertilizer used, N rate, and climatic conditions. Harris *et al.* (1994) found more applied fertilizer ((NH₄)₂SO₄) N loss during a drought year than during a normal one, although they note that this is

not usually the case. Olson *et al.* (1979) found 100 kg ha⁻¹ rate plots had twice as much fertilizer N that was unaccounted for as did the 50 kg ha⁻¹ rate plots. They also found most of the fertilizer remaining in the soil was in the top 0 - 10 cm layer of soil, with no sign of N below 50 cm. Thus, Olson *et al.* (1979) concluded N losses were not due to leaching, but to gaseous losses. Carter *et al.* (1967) stated that during drying procedures, N as ammonia was lost from samples where ammonium sulfate had been applied. Carter *et al.* (1967) concluded N was lost in the gaseous form throughout the year, but was more intense in the warmer months.

Researchers found varying levels of N loss. Harris *et al.* (1994) state that over a two year period, 39% of the total N applied was lost. Walters and Malzer (1990) found 7% of the applied fertilizer N was unaccounted. Reddy and Reddy (1993) found unaccounted N losses were 11 to 18% at 100 kg N ha⁻¹ and 34 to 48% for 200 kg N ha⁻¹. The unaccounted N was that not found in the plant or in the organic and inorganic fractions of the soil. This finding is consistent with the findings of Raun and Johnson (1995) where the soil-plant continuum buffers against nitrate accumulation in the soil profile. Hence, one would expect higher unaccountable N losses with higher N rates.

Carter *et al.* (1967) contributed some additional explanations for unaccounted N loss. Gaseous losses of N were higher during the warmer periods of the year (Carter *et al.*, 1967). Carter *et al.* (1967) explained some unaccounted losses by movement of nitrate into lower horizons where anaerobic conditions are more probable. Carter *et al.* (1967) also found that when

ammonium sulfate was used, significant losses of ammonia occurred when the samples were dried.

Unaccountable fertilizer N losses have been attributed to volatilization (both from the soil and from the plant), denitrification, and leaching. Webster *et al.* (1986) blamed denitrification processes for unaccounted N losses of 11% in the first year. Olson and Swallow (1984) attributed losses to leaching and denitrification; however, since only small amounts of fertilizer N were found in the lower depths, the researchers concluded that leaching was not a significant factor. Olson *et al.* (1979) found little evidence of leaching; therefore, they concluded that N losses must be from gaseous losses and not from leaching. They also found a direct relationship between the amount of fertilizer nitrate present in the topsoil and the amount of N unaccounted for. Olson *et al.* (1979) found no evidence of fertilizer applications causing a priming effect on mineralization rates. However, work by Westerman and Kurtz (1973) states the opposite.

Materials and Methods

Two long-term soil fertility experiments were selected for further study, #222 at Stillwater and #502 at Lahoma, Oklahoma. In the first year of this study, only experiment 222 was used. Experiment 222 was conducted on a Kirkland silt loam (fine, mixed, thermic Udertic Paleustoll). Experiments 222 and 502 are both long-term fertility trials that receive annual applications of N, P and K incorporated preplant in a conventional tillage continuous winter wheat

production system. The treatments selected for ^{15}N microplots were those where N rates varied, but annual P and K rates were constant. In the second year of this study, experiment 222 was continued and another was initiated, experiment 502 at Lahoma (Grant silt loam, fine, mixed thermic Typic Paleustoll), OK. Soil test levels at the initiation of each experiment are reported in Table 1.

The experimental design was a randomized complete block with 3 replications. Each plot measured 6.1 x 18.3 m. The area contained within the microplots was 32.8 x 76.2 cm. 'Tonkawa' winter wheat (*Triticum aestivum* L.) was sown at 31.8 kg ha⁻¹. After the wheat stand was established, 32.8 x 76.2 cm microplot metal frames were installed in the center of each plot. Each frame spanned four rows of wheat.

Microplots were fertilized using 11.8881 percent atom excess $^{15}\text{NH}_4^{15}\text{NO}_3$ at experiment 222 in November of 1995 and at experiment 502 in November of 1996. Experiment 222 was fertilized with non-labeled ammonium nitrate fertilizer in October of 1996. Fertilizer N was applied at rates of 0, 45, 90, and 135 kg N ha⁻¹ at experiment 222. Phosphorus and potassium were applied to all plots at fixed rates of 29 and 38 kg ha⁻¹, respectively. Fertilizer N was applied at rates of 0, 22, 45, 67, 90, and 112 kg N ha⁻¹ in experiment 502 with fixed rates of 20 kg P ha⁻¹ and 56 kg K ha⁻¹.

Soil cores, 2.22 cm in diameter, were taken to a depth of 250 cm in 1996 and to a depth of 120 m in 1997, and sectioned into 0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-90 cm, 90-120 cm, 120-150 cm, 150-180 cm, 180-210 cm, and

210-240 cm. The soil cores were refrigerated at 10°C prior to processing. Soil samples were ground to pass a 2 mm mesh sieve.

Forage at flowering and grain and straw samples were taken from each plot. $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ were determined in the forage samples following a 0.01 M calcium sulfate extraction using an automated flow injection analysis system (Lachat, 1989, 1990). The dilution factor was 100 for NH_4^+ and 10 for NO_3^- . Harvest was conducted by hand due to the small size of the microplots. Main plots were harvested using a Massey Ferguson 8XP research combine. Yield was calculated for the forage, grain, and straw samples. Total N in the soil, forage, grain and straw was determined using a Carlo Erba model NA 1500 dry combustion analyzer. ^{15}N in the soil, forage, grain and straw was determined using a stable isotope ratio mass spectrometer. ^{15}N atom percent excess was calculated, correcting for natural abundance. N loss was calculated as (total N accumulated at flowering) minus (total N accumulated in the grain and straw at harvest). Only the first 7 soil depths, down to 150 cm, were used to determine percent fertilizer N recovery in experiment 222 for 1996 as the latter depths contained insignificant amounts of ^{15}N .

Nitrogen use efficiency was determined following the calculations outlined in Moll *et al.* (1982). Statistical analysis was performed using SAS (SAS Institute, 1985) and Steel and Torrie (1980). The 5% significance level was used in all comparisons.

Two methods are commonly used to determine percent fertilizer recovery, both of which were used in this study. Using the difference method, percent

fertilizer recovery is equal to total N uptake in the fertilized plot minus total N uptake in the check plot divided by the rate of fertilizer applied. When using the isotope method, percent fertilizer recovery = $(100P(c-b))/(f(a-b))$; where P= total N in the plant part or soil in kg ha^{-1} , f= rate of ^{15}N fertilizer applied, a= atom percent ^{15}N in labeled fertilizer, b= atom percent ^{15}N in the plant part or soil receiving no ^{15}N , and c= atom percent ^{15}N in the plant part or soil that did receive ^{15}N .

Results and Discussion

Average monthly maximum and minimum temperatures and rainfall are reported for experiments 222 and 502 in Tables 2 and 3. Grain yield and fertilizer response are reported in Table 4 for both experiments. A significant linear increase in grain yield was observed at both sites and both years (experiment 222) with increasing N rate. Fertilizer response (kg grain per kg N applied) decreased with increasing N rate for experiment 222 in 1996 and experiment 502 in 1997.

Fertilizer N recoveries in wheat forage collected at flowering are reported in Table 5 for the isotope method and in Table 6 using the difference method. Using the isotope method, percent fertilizer N recovery was not significant at experiment 222 in 1996 or at experiment 502 in 1997. A linear trend was observed in 1997 at experiment 222 whereby fertilizer N recovery increased with increasing N rate. When calculated by the difference method, fertilizer N

recovery was not significant for N rate in 1997 at either experiment; but, response was linear at experiment 222 in 1996.

Plant N loss was calculated by subtracting grain and straw N uptake from forage N uptake determined at flowering (Table 7). N loss at experiment 222 ranged from a net gain of 12.05 kg N ha⁻¹ to a loss of 14.67 kg N ha⁻¹ for the 45 and 135 kg N rates, respectively, in 1996. In the following year, N losses ranged from a net gain of 11.82 kg N ha⁻¹, to a loss of 5.10 kg N ha⁻¹ for the 0 and 135 kg N ha⁻¹ plots, respectively. N loss at experiment 502 ranged from a gain of 20.48 to a loss of 41.55 kg N ha⁻¹. A linear trend was noted for N uptake in the grain, straw and forage for both years and experiments (Table 7). Increased loss (e.g., plant N volatilization) with increasing N applied supports the buffering concept as proposed by Raun and Johnson (1995) and Kanampiu et al. (1997) since losses other than leaching increased at the N rates greater than that required for maximum yield.

Percent fertilizer N recoveries from the isotope method were also used to calculate plant N loss (Table 8). Fertilizer N recovery in the grain and straw was subtracted from fertilizer N recovery in the forage to determine loss. Loss increased with increasing N rate at experiment 222 in 1996, but was inconsistent for both experiments in 1997 (Table 8). This method is probably an inaccurate estimate of plant N loss, because it does not account for N that has been mineralized from the soil organic pool.

Thirdly, plant N loss was determined using percent recovery calculated using the difference method (Table 9). This analysis indicated that plant N loss

increased with increasing N rate at experiment 222 for 1996, but was inconsistent for experiments 222 and 502 in 1997. This method may also be faulty because it assumes mineralization rates in the check and treated plots are the same.

Total fertilizer N recovery was estimated by summing the amounts found in the grain and straw from 1996 and 1997 at experiment 222 and from 1997 at experiment 502 and that found in the soil in 1997. This estimate does not account for N potentially lost via leaching, denitrification or through the plant as gaseous NH_3 . Even still, estimated total fertilizer N recovery was 86 and 66% at experiments 222 and 502, respectively (Table 10).

Fertilizer N recovery was not affected by N rate for the grain at experiment 222 in 1996 nor for the grain and straw in 1997 at experiment 222 and 502 (Table 10). A linear trend for fertilizer N recovery to decrease with increasing N rate was observed for straw in 1996 at experiment 222. Fertilizer N recovery in the soil (sum of amounts found within individually analyzed depths) decreased with increasing N applied at both locations.

Total fertilizer N recovery was higher for experiment 222 than 502 when evaluated for the same N rates (45 and 90 kg N ha⁻¹). This contradicts other research done at Oklahoma State University where total fertilizer N recovery was higher in 502 than 222 (unpublished data). However, this may be due to only one year of data present for experiment 502 in the current study.

Table 11 shows fertilizer N recovery by the difference method. Soil cannot be estimated since dry combustion is not a reliable estimate of soil total

N (random error is equal to $\pm 224 \text{ kg N ha}^{-1}$). Fertilizer N recovery was not affected by N rate for the grain and straw in 1996 at experiment 222 nor for the straw in 1997. A linear trend was observed for grain in 1997 at experiment 222 where fertilizer N recovery increased with increasing N rate. At experiment 502 in 1997, a linear trend for fertilizer N recovery to decrease with increasing N rate was observed for straw.

Total fertilizer N recovery by the isotope method does not account for N potentially lost via leaching, denitrification, or through the plant as gaseous ammonia. The lowest fertilizer N recovery, both overall and in the soil, was found in the 90 kg N ha^{-1} . Figures 1 and 2 depict fertilizer N recovery in the soil by depth for experiment 222 and 502, respectively. In all cases, more than half of the fertilizer was found in the first 30 cm. At those depths beyond 30 cm, all had accumulated about the same amount of fertilizer. At depths $> 150 \text{ cm}$, very little ^{15}N above natural abundance was present at experiment 222 in 1996.

Harper et al. (1987) documented that approximately 21% of applied fertilizer N was lost from wheat plants as NH_3 . Because of this we were interested in analyzing forage tissue at flowering for the presence of NH_4^+ . Combined with NO_3^- it was thought that the relationship between the two inorganic N forms would demonstrate whether or not the plant had an excess and if reduction was taking place. The ratio of NO_3^- to NH_4^+ was significant for N rate at experiment 222 in 1996 and at experiment 502 in 1997. Correlation between $\text{NO}_3^-:\text{NH}_4^+$ and N loss at maturity was low at experiment 222 in 1996, but was highly correlated ($R^2 = 0.47$) in 1997. At experiment 502 in 1997 the

$\text{NO}_3^-:\text{NH}_4^+$ ratio was also correlated with N loss ($R^2 = 0.29$). In 1997 at both experiments, NO_3^- concentration at flowering was related to N loss at maturity. As the $\text{NO}_3^-:\text{NH}_4^+$ increased, N loss increased (Figure 3).

Conclusion

Plant N loss plays a significant role in the efficiency of use of fertilizer N. In grain production systems, plant N loss is tied closely to N rate. As such, efforts should be made to minimize the amount of N fertilizer applied beyond the plant's needs. Maximum nitrogen use efficiency generally takes place at low N rates and prior to the rate required for maximum yield.

This work showed that loss of N from the plant and soil increased with increasing N applied in two studies employing the use of ^{15}N . Fertilizer N recovery accounting for ^{15}N removed in the grain and straw and that remaining in the soil at the end of the experiment decreased with increasing N applied, which was consistent with increased N loss (plant volatilization and denitrification) with increasing N applied. Wheat was found to accumulate up to 190 kg N ha^{-1} in the forage by flowering, yet only 150 kg N ha^{-1} could be accounted for in the grain and straw at maturity. The ratio of NO_3^- to NH_4^+ in wheat forage at flowering was found to be correlated with estimated plant N loss. This may serve as a method of identifying potential plant N loss in order to increase N use efficiency via alternative management strategies.

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Table 1. Treatments and surface soil test characteristics (0-15 cm) for experiments 222 and 502.

Experiment	Fertilizer Applied			pH	Soil Test Level			Organic C	Total N
	N	P	K		P	K			
	-----kg ha ⁻¹ yr ⁻¹ -----				mg kg ⁻¹	mg kg ⁻¹	g kg ⁻¹	g kg ⁻¹	
222	0	29	38	6.0	57	221	8.2	0.6	
	45	29	38	5.7	65	283	8.9	0.7	
	90	29	38	5.4	57	253	9.8	0.8	
	135	29	38	5.2	56	220	9.7	0.8	
502	0	20	56	5.7	57	417	4.6	0.9	
	23	20	56	5.7	50	373	5.1	0.9	
	45	20	56	5.6	65	409	4.3	0.7	
	67	20	56	5.5	58	389	4.5	0.9	
	90	20	56	5.4	52	426	4.3	0.7	
	112	20	56	5.3	55	455	5.1	0.9	

pH, 1:1 soil:water, K and P, Mehlich III; Organic C (carbon) and Total N, dry combustion.

Table 2. Average maximum and minimum temperatures and monthly rainfall for experiment 222, 1995-1997.

Month	Maximum, °C	Minimum, °C	Rainfall, mm
		<u>1995-1996</u>	
September	39	3	111
October	31	-1	16
November	26	-7	2
December	23	-12	50
January	24	-17	1
February	34	-28	5
March	28	-14	24
April	31	-4	10
May	33	7	48
Total			267
		<u>1996-1997</u>	
September	35	6	128
October	28	2	66
November	26	-7	74
December	24	-12	0
January	26	-18	6
February	22	-8	89
March	33	-4	23
April	29	-4	137
May	33	3	62
Total			585

Table 3. Average maximum and minimum temperatures and monthly rainfall for experiment 502, 1996-1997.

Month	Maximum, °C	Minimum, °C	Rainfall, mm
		<u>1996-1997</u>	
September	35	7	155
October	29	3	69
November	23	-6	66
December	22	-13	7
January	23	-15	5
February	21	-6	86
March	29	-6	14
April	27	-6	163
May	33	5	92
Total			657

Table 4. Grain yield from experiment 222, 1995-1997 and experiment 502, 1996-1997

Experiment	Fertilizer Applied			Grain Yield		Fertilizer Response ^p	
	N	P	K	1996	1997	1996	1997
	-----kg ha ⁻¹ yr ⁻¹ -----			-----kg ha ⁻¹ -----			
222	0	29	38	815.90	942.85	-	-
	45	29	38	1006.18	888.32	22.36	19.74
	90	29	38	1139.69	1135.40	12.66	12.62
	135	29	38	1235.64	1927.44	9.15	14.28
SED ^δ				62.96	128.10		
N rate linear				***	***		
N rate quadratic				ns	**		
502	0	20	56		1342.39		-
	23	20	56		1969.17		85.62
	45	20	56		2271.61		50.48
	67	20	56		2590.99		38.67
	90	20	56		3169.02		35.21
	112	20	56		4009.41		35.80
SED ^δ					582.33		
N rate linear					***		
N rate quadratic					ns		

^p kg grain per kg N applied

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

^δ SED = standard error of the difference between two equally replicated treatment means.

Table 5. Fertilizer N recovery in wheat forage collected at flowering for experiments 222 and 502, 1995-1997, isotope method.

Experiment	Fertilizer Applied			Fertilizer N Recovery ^a	
	N	P	K	1996 Forage	1997 Forage
	kg ha ⁻¹ yr ⁻¹			%	
222	0	29	38	-	-
	45	29	38	19.43	1.27
	90	29	38	21.51	3.24
	135	29	38	25.68	3.35
	SED ^b			4.17	0.46
N rate linear			ns	*	
N rate quadratic			ns	ns	
502	0	20	56	-	-
	23	20	56		17.41
	45	20	56		24.69
	67	20	56		28.11
	90	20	56		27.19
	112	20	56		25.52
SED ^b				3.82	
N rate linear				ns	
N rate quadratic				ns	

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

^b SED = standard error of the difference between two equally replicated treatment means.

^a forage samples collected at flowering

Table 6. Fertilizer N recovery in forage for experiments 222 and 502, 1995-1997, difference method.

Experiment	Fertilizer Applied			Fertilizer N Recovery [§]	
	N	P	K	1996 Forage	1997 Forage
	-----kg ha ⁻¹ yr ⁻¹ -----			-----%-----	
222	0	29	38	-	-
	45	29	38	20.42	53.44
	90	29	38	45.91	86.51
	135	29	38	54.14	92.48
SED [§]				11.62	17.08
N rate linear				*	ns
N rate quadratic				ns	ns
502	0	20	56	-	-
	23	20	56		121.58
	45	20	56		218.88
	67	20	56		153.21
	90	20	56		169.81
	112	20	56		144.98
SED [§]					41.13
N rate linear					ns
N rate quadratic					ns

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

§ SED = standard error of the difference between two equally replicated treatment means.

§ forage samples collected at flowering

Table 7. Forage, grain and straw N uptake and estimated plant N loss, experiments 222, 1996-1997, and 502, 1997

Location	Fertilizer Applied			Total N Uptake							
	N	P	K	1996			1997				
	kg ha ⁻¹ yr ⁻¹			Forage	Grain	Straw	Loss/ Gain ^δ	Forage	Grain	Straw	Loss/ Gain ^δ
				kg N ha ⁻¹							
222	0	29	38	29.40	23.47	12.74	-6.81	18.76	22.54	8.04	-11.82
	45	29	38	38.59	32.10	18.54	-12.05	42.81	23.13	21.43	-1.75
	90	29	38	70.72	40.63	27.50	2.59	96.62	31.01	55.02	-6.32
	135	29	38	102.49	48.41	39.41	14.67	143.61	51.69	71.93	5.1
	SED ^δ			8.20	4.40	2.79		19.91	2.90	11.91	
N rate linear				***	**	***		**	***	**	
N rate quadratic				ns	ns	ns		ns	**	ns	
502	0	20	56					29.46	32.83	11.08	-14.45
	23	20	56					56.21	50.01	26.68	-20.48
	45	20	56					127.96	57.05	47.54	23.37
	67	20	56					132.12	63.56	40.15	28.41
	90	20	56					182.29	90.54	63.05	28.70
	112	20	56					191.84	105.39	44.90	41.55
SED ^δ							24.79	14.65	9.55		
N rate linear								***	***	***	
N rate quadratic								ns	ns	*	

^δLoss/gain determined by subtracting forage N uptake at flowering from total N in the grain and straw at maturity.

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

^δ SED = standard error of the difference between two equally replicated treatment means.

Table 8. Forage, grain and straw N uptake and estimated plant N loss by percent fertilizer recovery, isotope method, experiments 222, 1996-1997, and 502, 1997

Location	Fertilizer Applied			Fertilizer N Recovery							
	N	P	K	Forage	Grain	Straw	Loss/ Gain ⁵	Forage	Grain	Straw	Loss/ Gain ⁵
	-----kg ha ⁻¹ yr ⁻¹ -----			-----%-----							
222	0	29	38	-	-	-	-	-	-	-	-
	45	29	38	19.43	12.89	5.85	0.69	1.27	0.70	0.56	0.01
	90	29	38	21.51	9.71	4.55	7.25	3.24	1.07	1.87	0.30
	135	29	38	25.68	12.04	3.49	10.15	3.35	1.23	1.84	0.28
SED ⁵				4.17	2.06	0.78		0.46	0.27	0.59	
N rate linear				ns	ns	*		*	ns	ns	
N rate quadratic				ns	ns	ns		ns	ns	ns	
502	0	20	56					-	-	-	-
	23	20	56					17.41	11.72	7.39	-1.7
	45	20	56					24.69	9.56	9.77	5.36
	67	20	56					28.11	11.99	7.62	8.5
	90	20	56					27.19	10.59	7.36	9.24
	112	20	56					25.52	12.32	5.26	7.94
SED ⁵								3.82	2.43	2.79	
N rate linear								ns	ns	ns	
N rate quadratic								ns	ns	ns	

⁵Loss/gain determined by subtracting forage N uptake at flowering from total N in the grain and straw at maturity.

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

δ SED = standard error of the difference between two equally replicated treatment means.

Table 9. Forage, grain and straw N uptake and estimated plant N loss by percent fertilizer recovery, difference method, experiments 222, 1996-1997, and 502, 1997

Location	Fertilizer Applied			Fertilizer N Recovery							
	N	P	K	1996			1997				
	kg ha ⁻¹ yr ⁻¹			Forage	Grain	Straw	Loss/ Gain ⁵	Forage	Grain	Straw	Loss/ Gain ⁵
	-----			-----%							
222	0	29	38	-	-	-	-	-	-	-	-
	45	29	38	20.42	19.17	12.89	-11.64	53.44	1.32	29.76	22.36
	90	29	38	45.91	19.07	16.40	10.44	86.51	9.42	52.20	24.89
	135	29	38	54.14	18.47	19.75	15.92	92.48	21.60	47.32	23.56
	SED ^δ			11.62	6.97	7.42		17.08	4.01	15.40	
N rate linear			*	ns	ns		ns	**	ns		
N rate quadratic			ns	ns	ns		ns	ns	ns		
502	0	20	56					-	-	-	-
	23	20	56					121.58	74.69	67.87	-20.98
	45	20	56					218.88	53.82	81.04	84.02
	67	20	56					153.21	45.87	43.40	63.94
	90	20	56					169.81	64.13	57.75	47.93
	112	20	56					144.98	64.78	30.20	50.00
SED ^δ							41.13	30.03	17.01		
N rate linear							ns	ns	*		
N rate quadratic							ns	ns	ns		

⁵Loss/gain determined by subtracting forage N uptake at flowering from total N in the grain and straw at maturity.

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

δ SED = standard error of the difference between two equally replicated treatment means.

Table 10. Fertilizer N recovery in the grain, straw and soil for experiments 222 and 502, 1996-97, isotope method.

Experiment	Fertilizer Applied			Fertilizer N Recovery						
	N	P	K	1996		1997		Total Grain + Straw (2yrs)	1997 Soil	Total
kg ha ⁻¹ yr ⁻¹			Grain	Straw	Grain	Straw	%			
222	0	29	38	-	-	-	-	-	-	-
	45	29	38	12.89	5.85	0.70	0.56	20.0	66.02	86.02
	90	29	38	9.71	4.55	1.07	1.87	17.2	34.70	51.9
	135	29	38	12.04	3.49	1.23	1.84	18.6	25.04	43.64
	SED ^δ			2.06	0.78	0.27	0.59		14.95	
N rate linear			ns	*	ns	ns			*	
N rate quadratic			ns	ns	ns	ns			ns	
502	0	20	56			-	-	-	-	-
	23	20	56			11.72	7.39	19.11	47.29	66.40
	45	20	56			9.56	9.77	19.33	33.49	52.82
	67	20	56			11.99	7.62	19.61	28.61	48.22
	90	20	56			10.59	7.36	17.95	21.50	39.45
	112	20	56			12.32	5.26	17.58	22.03	39.61
SED ^δ					2.43	2.79		8.25		
N rate linear					ns	ns			*	
N rate quadratic					ns	ns			ns	

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

δ SED = standard error of the difference between two equally replicated treatment means.

Table 11. Fertilizer N recovery in the grain, straw and soil for experiments 222 and 502, 1996-97, difference method

Location	Fertilizer Applied			Fertilizer N Recovery					
	N	P	K	1996		1997		Total Grain + Straw (2 yrs)	
	kg ha ⁻¹ yr ⁻¹			Grain	Straw	Grain	Straw		
				%					
222	0	29	38	-	-	-	-	-	
	45	29	38	19.17	12.89	1.32	29.76	63.14	
	90	29	38	19.07	16.40	9.42	52.20	97.09	
	135	29	38	18.47	19.75	21.60	47.32	107.14	
	SED ^δ			6.97	7.42	4.01	15.40	33.80	
N rate linear				ns	ns	**	ns		
N rate quadratic				ns	ns	ns	ns		
502	0	20	56			-	-	-	
	23	20	56			74.69	67.87	142.56	
	45	20	56			53.82	81.04	134.86	
	67	20	56			45.87	43.40	89.27	
	90	20	56			64.13	57.75	121.88	
	112	20	56			64.78	30.20	94.98	
SED ^δ						30.03	17.01		
N rate linear						ns	*		
N rate quadratic						ns	ns		

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

δ SED = standard error of the difference between two equally replicated treatment means.

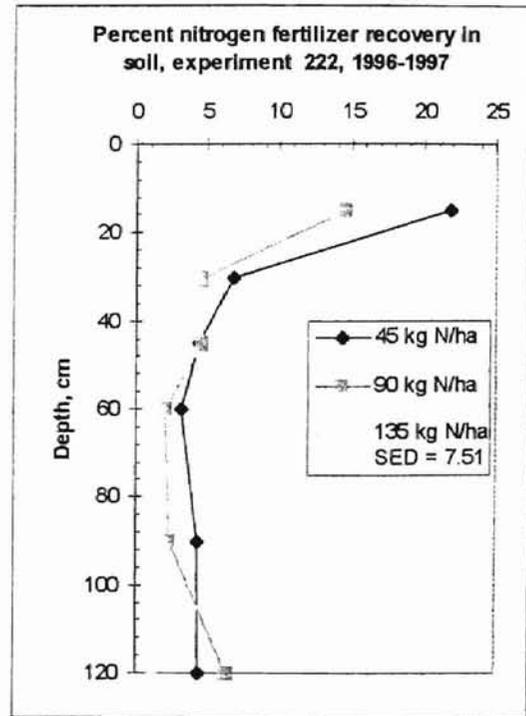
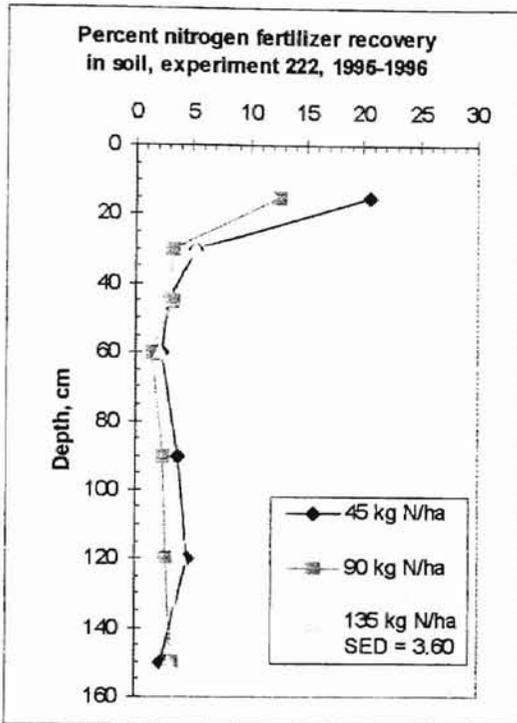


Figure 1. Fertilizer N recovery in the soil by depth and N rate, experiment 222, 1996 and 1997 (SED - standard error of the difference between two equally replicated means)

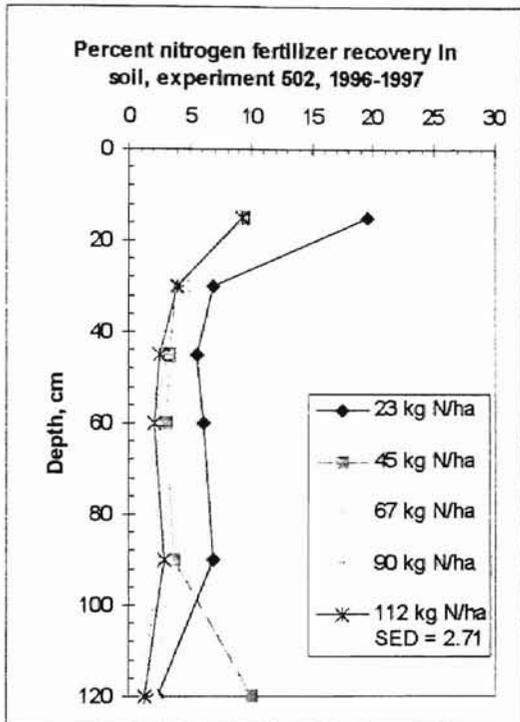


Figure 2. Fertilizer N recovery in the soil by depth and N rate, experiment 502, 1997 (SED - standard error of the difference between two equally replicated means)

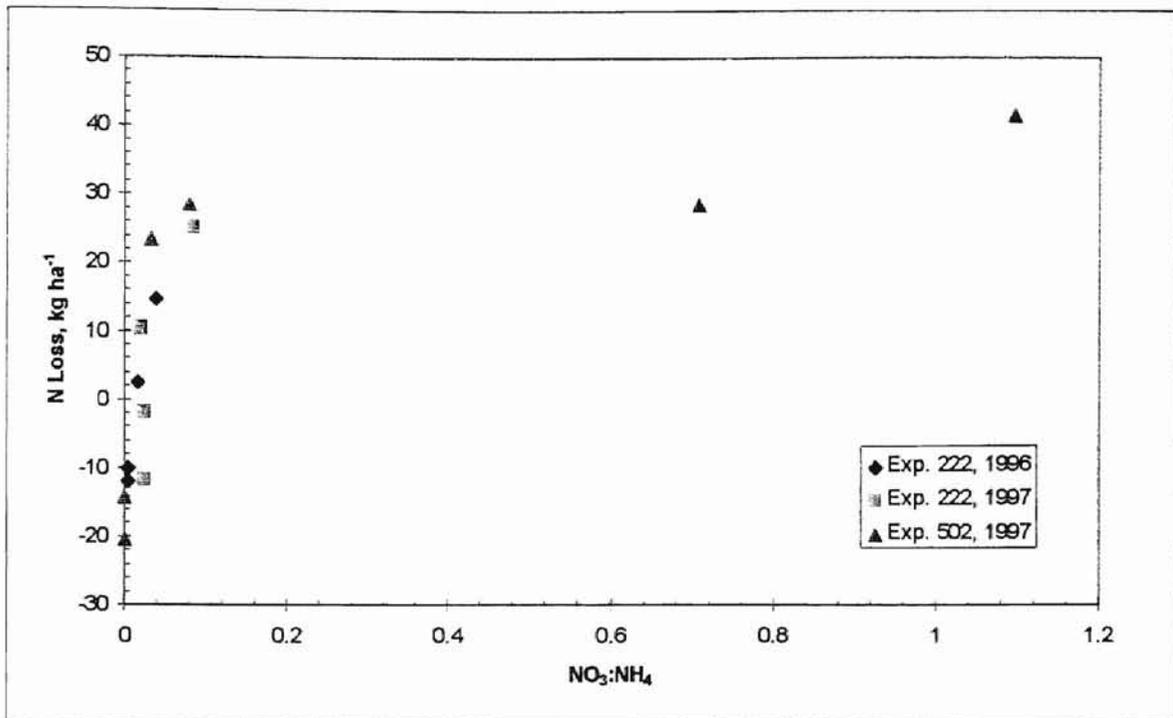


Figure 3. NO₃:NH₄ ratio versus N loss for experiments 222, 1996-1997, and 502, 1997

APPENDIX

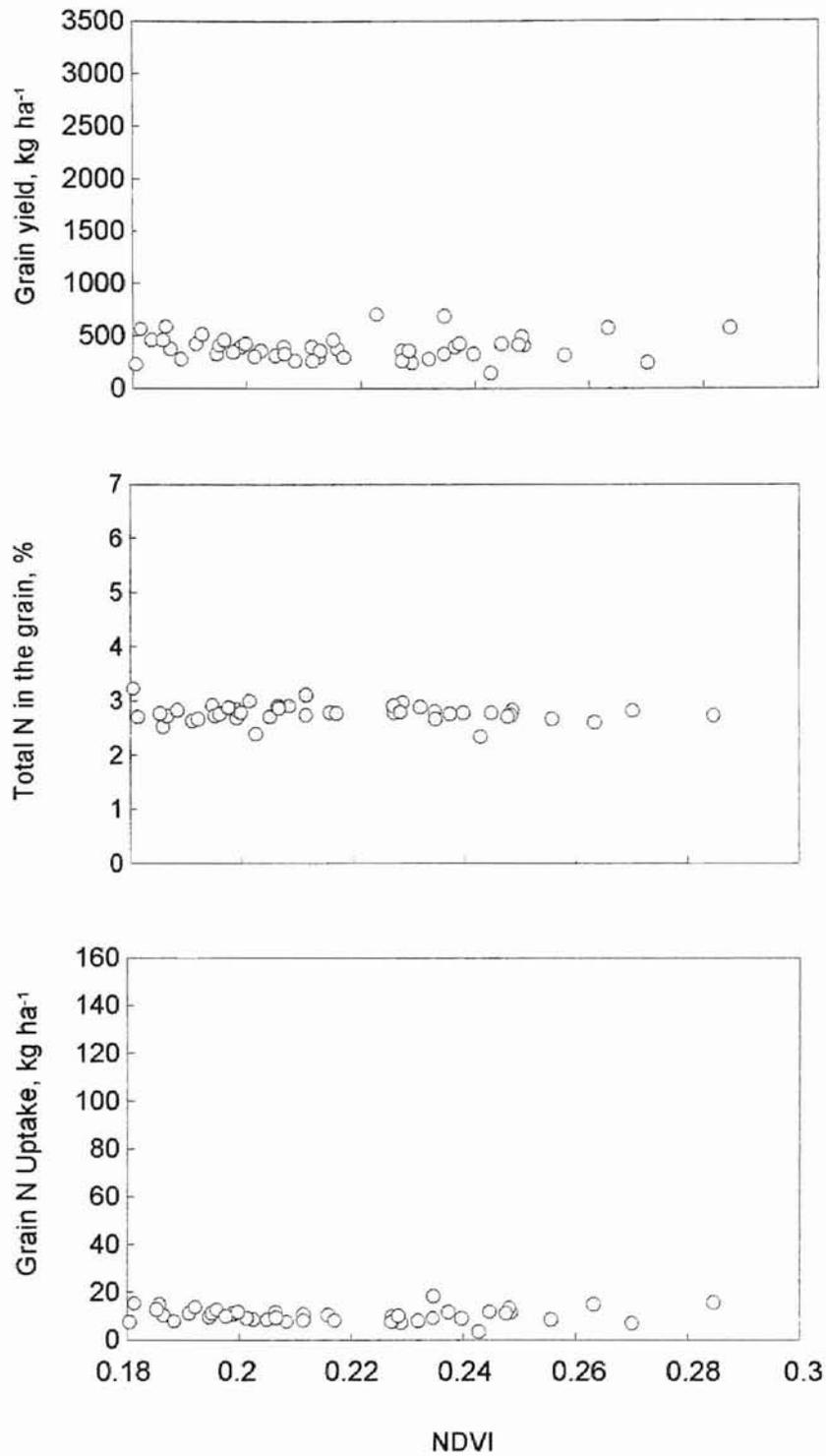


Figure 1. Correlation of grain yield, total N in the grain, and grain N uptake with NDVI (29 Mar 1996 Feekes growth stage 5) on variable rate plots, Altus, OK, 1996

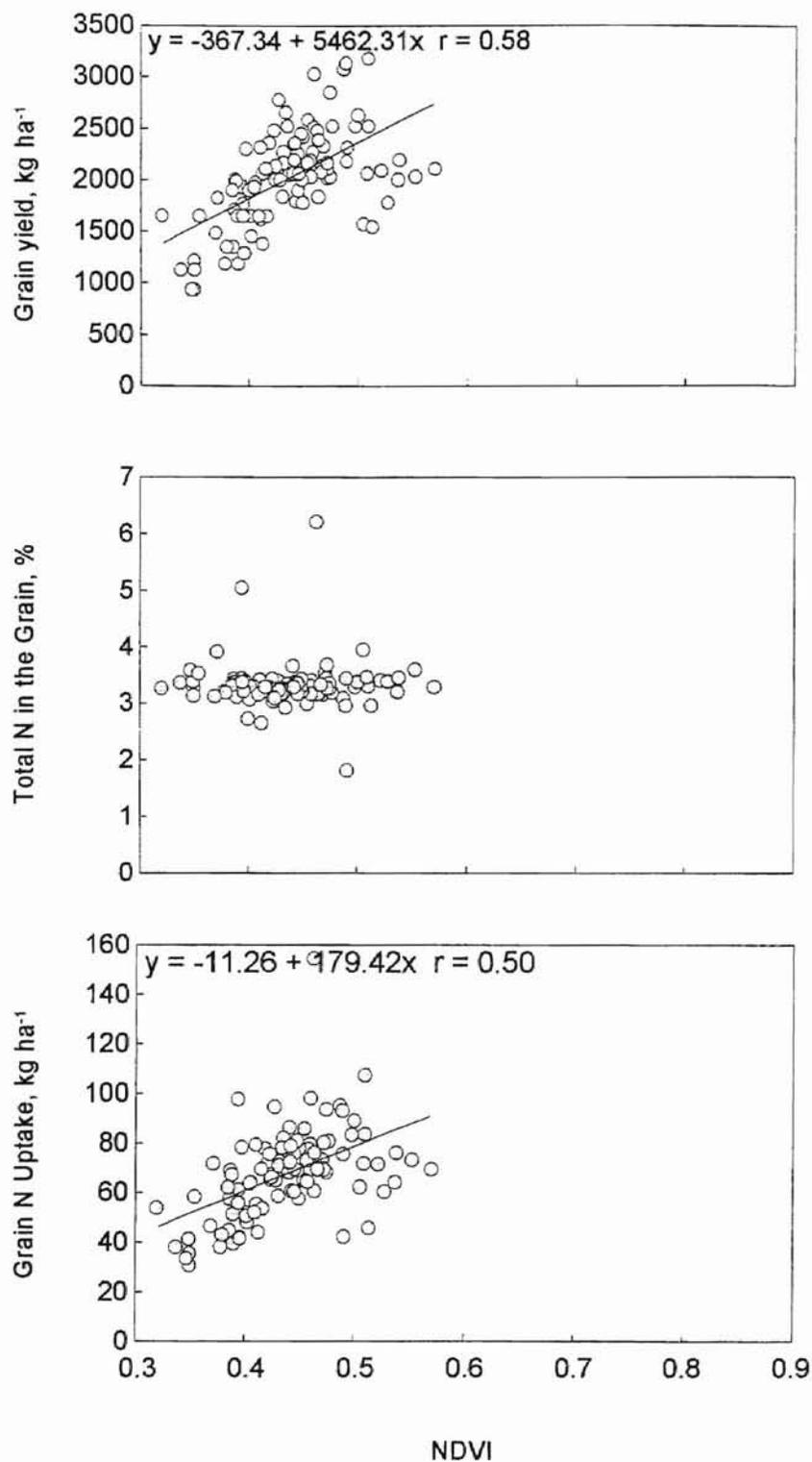


Figure 2. Correlation of grain yield, total N in the grain, and grain N uptake with NDVI (2 Apr 1996, Feekes growth stage 6) on variable rate plots, Hennessey, OK, 1996

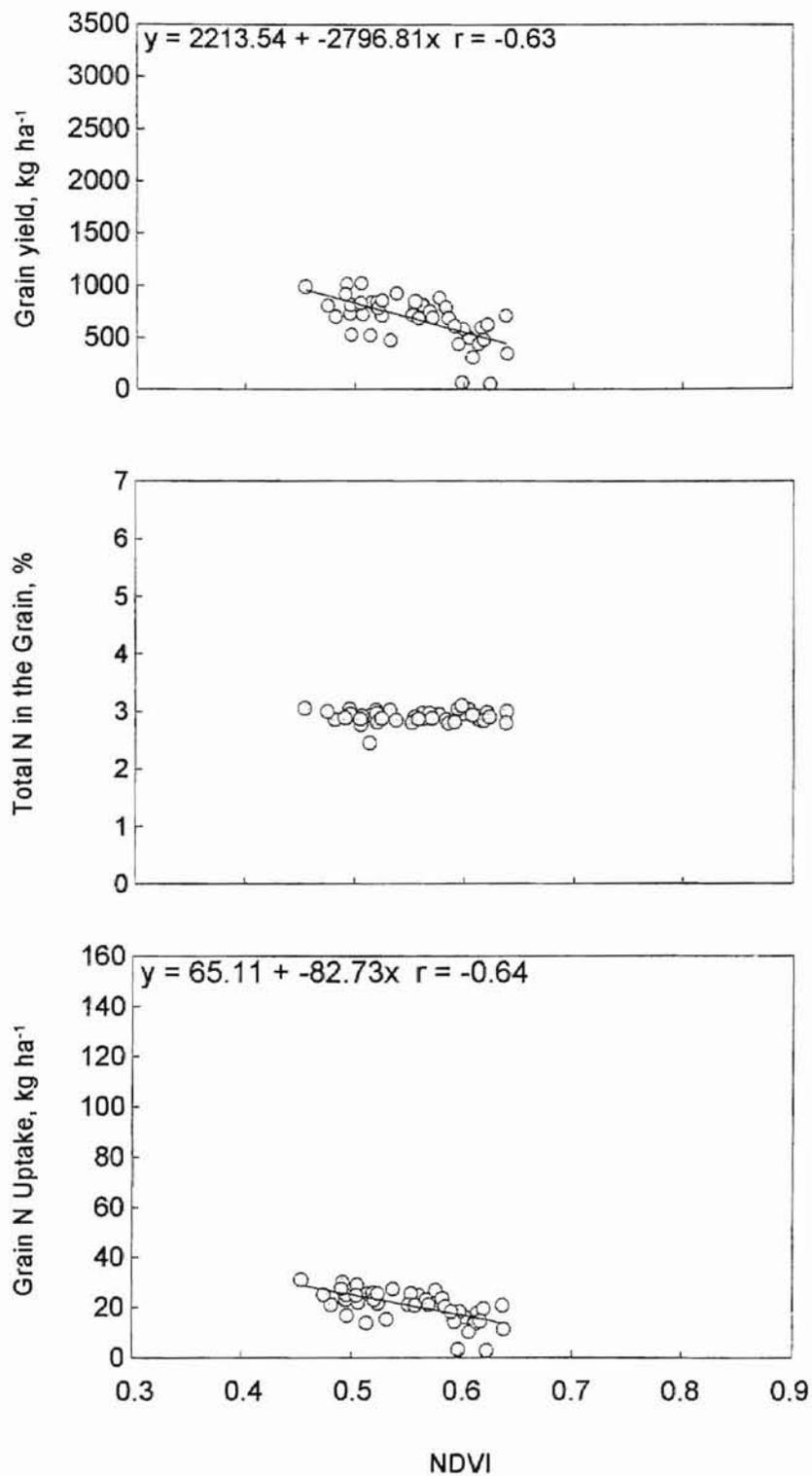


Figure 3. Correlation of grain yield, total N in the grain, and grain N uptake with NDVI (4 Feb 1997, Feekes growth stage 5) on variable rate plots, Tipton, OK, 1997

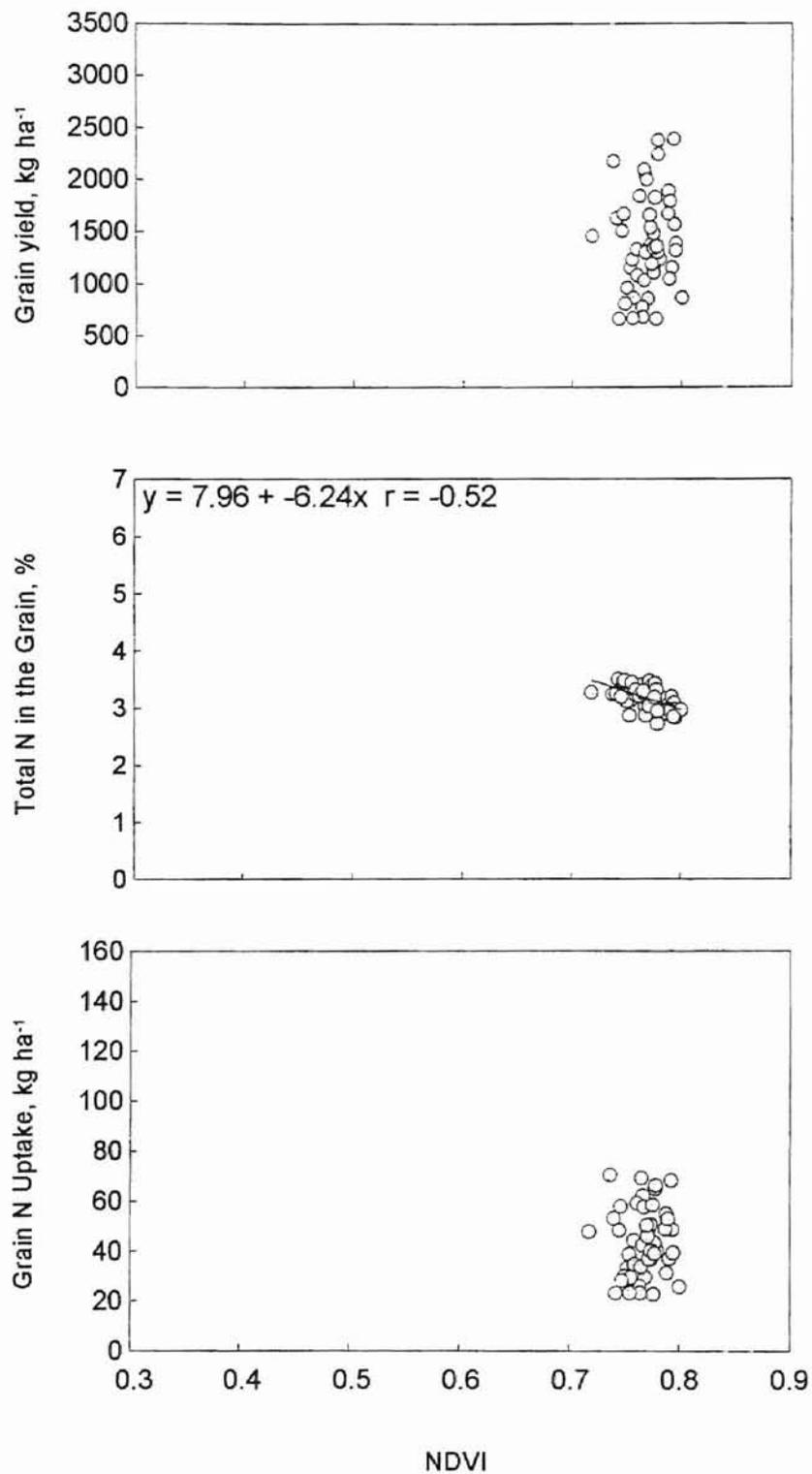


Figure 4. Correlation of grain yield, total N in the grain, and grain N uptake with NDVI (11 Feb 1997, Feekes growth stage 5) on variable rate plots, Hennessey, OK, 1997

Procedures for adding ^{15}N stock solution in the field.

1. Make-up stock solution of $^{15}\text{NH}_4^{15}\text{NO}_3$ containing 22.5000g of N/liter (64.3185 g of $^{15}\text{NH}_4^{15}\text{NO}_3$ /liter).
2. Pipette the correct aliquot of ^{15}N stock solution into a 1 liter volumetric flask.
3. Bring up to final volume with double-distilled H_2O (1000ml).
4. Pour the ^{15}N solution into a receiving vessel.
5. Rinse the 1 liter volumetric with 2 250ml rinses of double-distilled H_2O and pour into receiving vessel.
6. Final volume in receiving vessel is 1000ml.
7. Broadcast the 1000ml ^{15}N solution uniformly over the 0.25m^2 area. Use sprayer.
8. Exercise extreme caution to prevent cross contamination of plots and to insure all of the ^{15}N material is applied uniformly.
9. The metal rectangle ($76.2 \times 32.8\text{cm} = 0.25\text{m}^2$) must be inserted prior to application of ^{15}N . Each rectangle must be equally placed across 4 drill rows in the center of each plot.

All other areas outside the ^{15}N -fertilizer area should be treated with non-labeled fertilizer.

Vita

Heather Lynn Lees

Candidate for the Degree of

Master of Science

Thesis: DEVELOPMENT OF FIELD STANDARDS FOR AN OPTICAL SENSOR
VARIABLE RATE APPLICATOR AND ESTIMATING GASEOUS
NITROGEN LOSSES FROM *TRITICUM AESTIVUM* L. USING ¹⁵N

Major Field: Agronomy

Biographical:

Personal Data: Born in Newark, Ohio on January 19, 1973, the daughter
of David Watson and Suzanne Allison Lees.

Education: Graduated from Northridge High School, Johnstown, Ohio in
June 1991; received Bachelor of Science degree in Agriculture
from Wilmington College, Wilmington, Ohio in May 1995.
Completed the requirements for the Master of Science degree in
Agronomy from Oklahoma State University in December 1997.

Experience: Employed by Oklahoma State University, Department of
Agronomy as a graduate research assistant in the Soil Fertility
Project, August 1995 to present.

Professional Memberships: American Society of Agronomy and Soil
Science Society of America.