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PRODUCTION SYSTEM TECHNIQUES TO INCREASE NITROGEN USE EFFICIENCY IN WINTER WHEAT*

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ABSTRACT

Most current research on winter wheat (*Triticum aestivum* L.) focuses on increasing yields of either grain or plant biomass. Increased production costs and environmental awareness will promote the development of methods to increase the efficiency of applied nutrients. Nitrogen (N) is often the most limiting nutrient for cereal grain production and represents one of the highest input costs in agricultural systems. This study was conducted to evaluate the effects of several short-term practices on nitrogen use efficiency (NUE) in winter wheat at three locations in Oklahoma. The variables evaluated included variety, nitrogen source, nitrogen timing, nitrogen rate, production system (forage only vs. grain only and a combination of the two), resolution of

2261

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THOMASON ET AL.

nitrogen application based on in-season estimated yield (INSEY), and application of a late-season senescence delaying chemical and late-season KH_2PO_4 . Results indicate that many approaches can be taken to increase NUE in wheat production systems. Averaged over 9 site yrs, the highest NUE was for forage-only production systems (66% for "Jagger" and 52% for "2174") far higher than grain-only production systems (26% for "Jagger" and 37% for "2174"). The combination of a 3-way split application using sensor measurements and 1 m² application resolution produced the highest average grain-only NUE at 81% for 2174 and 48% for Jagger compared with 29% NUE for pre-plant applied N. The most critical components of NUE from this study appear to be production system, variety, N fertilizer timing, and INSEY based topdress N applications.

INTRODUCTION

Nitrogen use efficiency is defined as grain production per unit of N available in the soil and calculated as grain weight divided by N supplied (Gw/Ns).^[1] In this study, we calculated NUE as uptake efficiency (the difference of N uptake in the treated plot and N uptake in the 0-N check, divided by the total applied N rate. Uptake efficiency from the soil is critical to the overall NUE of the system, therefore techniques that enhance uptake or provide N directly to the plant need to be developed and evaluated. Conversion of N to plant material and grain are both critical when considering increased NUE. A plant more efficient at converting N from the tissue to grain N will have increased NUE.

It has been noted that different NUEs among different corn hybrids are largely due to differing utilization of N already accumulated in the plant prior to anthesis, especially with low N levels.^[1] Eghball and Maranville^[2] found that NUE usually parallels water use efficiency in corn, thus the two traits can be selected simultaneously where such parallels exist. Wheat varieties with high harvest index values are known to have higher NUEs.^[3] It has been reported that wheat varieties that accumulate large amounts of N early in the growing season do not necessarily have high NUE. Plants must convert this accumulated N to grain nitrogen and must assimilate N after anthesis to produce high NUEs.^[4] Since most variety selection is done under high N fertility conditions, efficiency of N use is often considered second in importance to total yield. This approach will have to change in response to the worldwide need for more nutrient efficient crops.

In the south-central United States, producers often use winter wheat as a forage crop for cattle as well as for grain production. Research indicates that

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NUE IN WINTER WHEAT

forage production systems are more efficient users of N than grain production systems with forage system NUEs over 70% and grain-only NUEs less than 40%.^[5] Working in corn, O'Leary and Rehm^[6] reported that NUE values were greater for silage than those for grain. Much of the loss of applied N fertilizer efficiency is due to the loss of N to the atmosphere at senescence.^[7] At flowering, N is translocated to the grain and movement at this stage of development causes gaseous N losses to increase and efficiency to decrease.^[8]

Some researchers have noted that application of N as NH_4 will produce plants with higher total N uptake and therefore higher NUE. As stated earlier, late-season N uptake and assimilation are critical for increasing NUE. Nitrogen in the NH_4 form is not mobile in the soil and may therefore be available for lateseason uptake by the plant. Plants with preferential uptake of NH_4 during grain fill may provide increases in NUE over plants without this preference.^[9] Ammonium–N supplied to high yielding corn genotypes increased yield over plants supplied with NO₃ during critical ear development.^[10] Plant assimilation of NO₃ requires the equivalent of 20 ATP mol⁻¹ NO₃, but NH_4 assimilation requires only five ATP mol⁻¹ of NH_4 .^[11] It is evident that this energy savings could be beneficial to the plant late in the season.

Fertilizer use efficiency as reflected in grain yield of winter wheat has been shown to change with time and rate of application.^[12] Studies by Harper et al.^[8] noted decreased N concentrations in winter wheat with time during the growing season. Olson and Swallow^[13] noted in-season N application resulted in increased efficiency in four of five years when compared to pre-plant incorporated nitrogen in winter wheat. Nitrogen supplied late-season has been shown to increase grain protein and NUE over pre-plant applied nitrogen.^[14] In another study by Wuest and Cassman^[15] recovery of pre-plant N was found to be less than 55%, while recovery of N applied at anthesis was noted at 55–80%.

Precision agriculture practices can increase NUE by providing precise in-season application of N fertilizer. To capitalize on any potential N fertilizer savings and increased NUE, management decisions need to be made at the appropriate field element size.^[16–18] Field element size is defined as that area or resolution which provides the most precise measure of the available nutrient where the level of that nutrient changes with distance.^[17] Random variability in soil test and plant biomass has been documented at resolutions less than or equal to one square meter.^[16–18] When N management decisions are based on this information, the variability in the crop present at that resolution can be detected using optical sensors (normalized difference vegetative index or NDVI).^[17,19] Differences can then be addressed by supplying N at prescribed rates, thus increasing NUE.^[19]

Ethephon [(2-chloroethyl) phosphonic acid] applied at either Feekes growth stage 6 or 9 has shown increased N remobilization from vegetative plant parts and increased dry matter levels at harvest.^[20] Foliar applications of KH₂PO₄ at rates

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2264

THOMASON ET AL.

of 10 kg ha^{-1} have been shown to increase grain yields in regions where lateseason drought and temperature stress occurs.^[21] The objectives of this trial were to evaluate the effects of variety, nitrogen source, nitrogen timing, nitrogen rate, production system (forage only vs. grain only and a combination of the two), resolution of nitrogen application, and application of a late-season senescence delaying chemical and late-season KH₂PO₄ on NUE in winter wheat.

MATERIALS AND METHODS

Three experiments were conducted at Stillwater, OK on a Norge loam (finesilty, mixed, active, thermic Udic Paleustolls), Tipton, OK on a Tillman–Hollister sandy loam (fine, mixed, superactive, thermic Typic Paleustolls), and Haskell, OK on a Taloka silt loam (fine-mixed, thermic Mollic Albaqualf) (Table 1). The treatment variables were wheat variety, nitrogen source, nitrogen timing, nitrogen rate, production system (forage only vs. grain only and a combination of the two), resolution of nitrogen application, and application of a late-season senescence delaying chemical and late-season KH_2PO_4 . The effects of these treatments on NUE were evaluated, which in this case, was calculated as (N uptake in the treated plot–N uptake in the check plot)/total N rate applied. Two wheat varieties,

		NH ₄ –N	NO ₃ –N	P ^b	K ^b	Total N ^c	Organic C ^c
Location	$p\mathrm{H}^{\mathrm{a}}$		mg kg ⁻	1		m	gg^{-1}
Stillwater	6.2	2.2	5.6	28	472	0.09	1.06
Classifi	cation: N	orge loam (f	ìne-silty, mix	ked, ther	mic Udic	Argiustoll)
Tipton	7.4	23.6	5.6	85	1,006		
Classifi	cation: Ti	llman–Hollis thermi	ster sandy lo c Typic Pale	· ·	e, mixed, s	superactive	; ,
Haskell (pre-liming)	4.8	43.1	32.1	45	240		
Haskell (post-liming)	6.1	28.2	33.0	41	252		
u U	cation: Ta	loka silt loai	m (fine-mixe	d, thern	nic Mollic	Albaqual	f)

Table 1. Initial Soil Chemical Characteristics and Classification (0–15 cm) at Stillwater, Tipton, and Haskell, OK

^apH: 1:1 soil: water.

^bP and K: Meilich III.

^cOrganic C and total N: dry combustion.

NUE	IN WIN	NT I	ER	W	HE.	AT														2265
te, Topdress	Chemical													Ethephon	$\rm KH_2PO_4$					
Rate, Topdress N Ra	N at Flowering kg ha ⁻¹	0	0	0	0	22	22	22	22	22	22	0	0	22	22	0	0	22	22	
/stem, Pre-plant N F	TD Resolution kg ha ⁻¹					1 m	1 m		1 m	1 m		1 m		1 m	1 m	1 m	1 m	1 m	l m	bove 4.4°C.
at Production Sy	TD N Rate kg ha ⁻¹	0	0) O	0	INSEY	INSEY	78	INSEY	INSEY	45	INSEY	78	INSEY	INSEY	INSEY	INSEY	INSEY	INSEY	rrier (2 L). 4. 1 average temp al
ety, Winter Whe	PP N Rate kg ha ⁻¹	0	117	0	112	0	0	0	34	34	34	0	0	0	0	56	56	56	56	
Table 2. Treatment Structure Including Variety, Winter Wheat Production System, Pre-plant N Rate, Topdress N Rate, Topdress Resolution, and N Applied at Flowering, 1998–2001	Production System	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Forage	Forage	Forage & grain	Forage & grain	All N rates applied as actual N in kg ha ⁻¹ . Topdress resolution is square meters. Ethephon applied at Feekes 9 at 0.42 kg ai ha ⁻¹ . KH ₂ PO ₄ applied at anthesis at 10 kg material ha ⁻¹ in water carrier (2 L). Variety 2180 was planted in crop year 1998–99 in lieu of 2174. INSEY: NDVI at Feekes 5 divided by days from planting with average temp above 4.4°C.
Freatment Struard Applie	Variety	2174	2174	Jagger	Jagger	2174	Jagger	2174	2174	Jagger	2174	2174	2174	2174	Jagger	2174	Jagger	2174	Jagger	All N rates applied as actual N in kg Fopdress resolution is square meters. Ethephon applied at Feekes 9 at 0.42 KH ₂ PO ₄ applied at anthesis at 10 kg Variety 2180 was planted in crop yet NSEY: NDVI at Feekes 5 divided b
Table 2. Resolution,	Treatment	-	- c	1 ო	4	5	9	7	8	6	10	11	12	13	14	15	16	17	18	All N rates Topdress re Ethephon a KH2PO4 at Variety 218 INSEY: NI

NUE IN WINTER WHEAT

2265

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2266

THOMASON ET AL.

2174 and Jagger were planted in plots with pre-plant N rates of 0, 34, 56, or 112 kg ha^{-1} as ammonium nitrate (34–0–0) in the 1999–00 crop year. Variety "2180" was planted instead of 2174 in 1998. Two treatments received fixed-rate topdress N applications of $78 \text{ kg} \text{ ha}^{-1}$ and one treatment received $45 \text{ kg} \text{ N} \text{ ha}^{-1}$, with eleven others receiving a prescribed topdress N rate based on NDVI readings and INSEY values.^[22,23] The INSEY index is computed by taking one NDVI reading between Feekes growth stages 4 and 6,^[24] and dividing by the number of days from planting to the date the reading was taken with average temperature daily above 4.4°C. The plots receiving N based on INSEY values were sensed and treated on a 1 m^2 resolution, while plots receiving fixed rates of N were fertilized on a whole plot basis (13.9 m²). Variable rates were applied at a range of 0-78 kg N ha⁻¹. Ten treatments, some with variable and some with fixed topdress rates received an additional 22 kg N ha^{-1} as urea ammonium nitrate (28–0–0) at flowering. Two treatments, one for each variety, were grown for forage-only with forage removed at Feekes growth stage 5 and again at flowering. Two treatments, again one for each variety, were managed for both forage and grain with only one forage harvest at Feekes growth stage 5. A late-season senescence-delaying chemical, Ethephon (CAS# 16672-87-0), was applied to one treatment at Feekes 9 to attempt to increase nitrogen use efficiency (Table 2). Potassium dihydrogen phosphate (KH₂PO₄) was applied at a rate of 10 kg ha⁻¹ of material in 2 L of H₂O at flowering to one treatment.

Forage samples from forage-only, and forage + grain plots were harvested from 1 m^2 areas in the center of the plots at Feekes 5 and the entire plot was mowed to a height of 15 cm. In the forage-only plots, forage was again harvested from 1 m^2 in the center of the plot at flowering. In the forage + grain plots, forage was harvested from a 1 m^2 area in the center of the plot at Feekes 5 and the plot was then mowed but allowed to re-grow and produce grain. Forage harvests were taken by hand at both growth stages. From all grain and forage + grain plots, grain was harvested from an area of $3.05 \times 2 \text{ m}$ using a self-propelled combine. Forage and grain samples were dried and ground to pass a 140 mesh sieve (100 um) and analyzed for total N content using a Carlo-Erba NA 1500 automated dry combustion analyzer.^[25] Statistical evaluation and analysis of variance was performed using SAS.^[26] Nitrogen use efficiency was calculated by subtracting the yield of the unfertilized check from the yield of the fertilized plot and then dividing by the total N rate applied.

RESULTS

Results will be presented as individual years and locations with grain data following in Tables 3–5, forage-only data in Table 6 and data from forage and grain plots in Table 7.

NUE I	N WINTE	ER WHEAT	2267
Rate, NUE;	Chemical	KH ₂ PO ₄ KH ₂ PO ₄	(continued)
Rate, Total N	Production System	Grain Grain Grain Grain Grain Grain Grain Grain	Grain
Flowering N	Variety	Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger Jagger	Jagger
N Rate, I Jipton, OK	%	$\begin{vmatrix} & 2 & 2 \\ - & 2 & - \\ - & - & - \\ - & - & - \\ - & - & -$	71
te, Pre-plant askell, and T	Total N Rate kg ha ⁻¹	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	112
Table 3. Treatment; Mean Grain Yield, Grain N Uptake, Topdress N Rate, Pre-plant N Rate, Flowering N Rate, Total N Rate, NUE; Variety, Production System, and Chemical Application, 1999, Stillwater, Haskell, and Tipton, OK	Flowering N kg ha ⁻¹	Stillwater 0 0 22 22 22 22 22 22 0 0 0 0 1 Tipton 0 0 0 0 0 0 0 0 0 0 0 0 0	0
in N Uptake, ' plication, 199	Winter Topdress kg ha ⁻¹	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0
in Yield, Grai Chemical Ap	Pre-plant N kg ha ⁻¹	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	112
t; Mean Gra System, and	N Uptake kg ha ⁻¹	60 85 85 85 86 86 86 85 85 85 85 85 85 85 85 85 85 85 85 85	96
Treatment; Production Sy	Yield kg ha ⁻¹	2,282 2,091 1,687 3,211 3,211 2,173 1,832 392 1,932 1,971 1,777 1,777 1,777 1,976 1,939 1,059 1,956 2,951 719	2,960
Table 3. Variety, 1	Trt	3 SED 8 SED 9 6 6 4 3 3 8 9 6 6 4 4 3 3 8 9 6 6 6 7 3 9 8 1 1 8 1 8 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	4

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Chemical									Ethephon	$\rm KH_2PO_4$				
Production System	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	f_g	f_g		
Variety	2180	Jagger	2180	2180	Jagger	2180	2180	2180	2180	Jagger	2180	Jagger		
NUE %	15	61	21	93	101	76	- 1	17	11	62	34	16	4	
Total N Rate kg ha ⁻¹	59	57	100	69	70	56	31	78	57	61	100	90		us.
Flowering N kg ha ⁻¹	22	22	22	22	22	22	0	0	22	22	22	22		of the difference between two equally replicated means. Feekes 9 at $0.42 \text{ kg ai } \text{ha}^{-1}$. roduction system. induesis at 10 kg material ha^{-1} in water carrier (2 L).
Winter Topdress kg ha ⁻¹	37	35	78	13	14	0	31	78	35	39	22	12		r of the difference between two equally replicated me : Feekes 9 at $0.42 \text{ kg ai } ha^{-1}$. production system. anthesis at 10 kg material ha^{-1} in water carrier (2 L).
Pre-plant N kg ha ⁻¹	0	0	0	34	34	34	0	0	0	0	56	56		of the difference between Feekes 9 at 0.42 kg ai ha ⁻¹ roduction system. inthesis at 10 kg material h
N Uptake kg ha ⁻¹	51	51	63	106	87	76	41	55	48	64	76	56	11	r of the difference t Feekes 9 at 0.42 k production system. anthesis at 10 kg m
Yield kg ha ⁻¹	2,090	1,924	2,443	3,842	3,038	3,361	1,991	2,324	2,098	2,222	2,568	1,774	412	SED: Standard error Ethephon applied at f_g: forage + grain pi KH ₂ PO ₄ applied at a
Trt	5	9	7	8	6	10	11	12	13	14	17	18	SED	SED: S Ethephc f_g: foi KH ₂ PO

THOMASON ET AL.

Table 3. Continued

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NUE IN	N WINTEI	R WH	IEA	Г																	2	269
Rate, NUE;	Chemical													Ethephon	$\rm KH_2PO_4$							(continued)
Table 4. Treatment; Mean Grain Yield, Grain N Uptake, Topdress N Rate, Pre-plant N Rate, Flowering N Rate, Total N Rate, NUE; Variety, Production System, and Chemical Application, 2000, Stillwater, Haskell, and Tipton, OK	Production System	G.orin	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	f b	f b			Grain	Grain	
Flowering N , OK	Variety	171 C	2174 2174	Jagger	Jagger	2174	Jagger	2174	2174	Jagger	2174	2174	2174	2174	Jagger	2174	Jagger			2174	2174	
t N Rate, id Tipton	%		38		37	22	46	37	48	59	55	45	36	40	38	12	24	23			18	
tte, Pre-plant , Haskell, ar	Total N Rate kg ha ⁻¹	0	112	0	112	59	57	100	69	70	56	31	78	57	61	100	90			0	112	
Table 4. Treatment; Mean Grain Yield, Grain N Uptake, Topdress N Rate, Pre-plant N Rate, Flow Variety, Production System, and Chemical Application, 2000, Stillwater, Haskell, and Tipton, OK	Flowering N kg ha ⁻¹	Stillwater	0 0	0	0	22	22	22	22	22	22	0	0	22	22	22	22		Haskell	0	0	
iin N Uptake, Application, 20	Winter Topdress kg ha ⁻¹	c	0 0	0	0	37	35	78	13	14	0	31	78	35	39	22	12			0	0	
ain Yield, Gra d Chemical A	Pre-plant N kg ha ⁻¹	c	112	0	112	0	0	0	34	34	34	0	0	0	0	56	56			0	112	
nt; Mean Gr System, an	N Uptake kg ha ⁻¹	75	<i>در</i> 117	52	93	88	78	112	108	93	106	89	103	98	75	87	74	11		51	71	
Production	Yield kg ha ⁻¹	2 205	3,894	2,816	2,925	3,498	3,370	4,135	3,873	3,290	3,792	3,921	4,248	3,894	3,552	3,031	2,911	295		1,935	1,938	
Table 4 Variety,	Trt	-	- 7	б	4	5	9	7	8	6	10	11	12	13	14	17	18	SED		1	7	

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2270

THOMASON ET AL.

	Chemical									Ethephon	$\rm KH_2PO_4$			
	Production System	Grain Grain	Grain	Grain Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	f g_f	f g_f	
	Variety	Jagger Jagger	2174	Jagger 2174	2174	Jagger	2174	2174	2174	2174	Jagger	2174	Jagger	
	NUE %	s	16	63 2	26	8	32	14	15	19	1		-14	11
ned	Total N Rate kg ha ⁻¹	0 112	57	61 22	85	86	56	41	78	56	62	106	105	
Table 4. Continued	Flowering N kg ha ⁻¹	0 0	22	22 22	22	22	22	0	0	22	22	22	22	
Та	Winter Topdress kg ha ⁻¹	0 0	35	39 0	29	30	0	41	78	34	40	28	27	
	Pre-plant N kg ha ⁻¹	0 112	0	0 0	34	34	34	0	0	0	0	56	56	
	N Uptake kg ha ⁻¹	36 30	09	37 65	73	43	69	56	63	62	37	50	21	9
	Yield kg ha ⁻¹	$1,180\\780$	2,042	1,088 1.955	2,223	1,168	1,909	2,005	2,033	2,082	1,089	1,439	572	215
	Trt	ω4	Ś	9 10	8	6	10	11	12	13	14	17	18	SED

		-											ephon	$\rm KH_2PO_4$				
													Eth	KH				
	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	f 8	ц в		
	2174	2174	Jagger	Jagger	2174	Jagger	2174	2174	Jagger	2174	2174	2174	2174	Jagger	2174	Jagger		
		40		57	37	30	40	92	118	64	40	40	39	62	25	20	13	
	0	112	0	112	43	40	100	65	60	101	20	78	41	39	88	82		eans.
Tipton	0	0	0	0	22	22	22	22	22	22	0	0	22	22	22	22		SED: Standard error of the difference between two equally replicated means. Ethephon applied at Feekes 9 at 0.42 kg ai ha^{-1} . E.g: forage + grain production system. KH ₂ PO ₄ applied at anthesis at 10 kg material ha^{-1} in water carrier (2 L).
	0	0	0	0	20	18	78	6	4	45	20	78	19	17	10	4		n two equal -1. . ha ⁻¹ in wa
	0	112	0	112	0	0	0	34	34	34	0	0	0	0	56	56		SED: Standard error of the difference betwee 5thephon applied at Feekes 9 at 0.42 kg ai ha <u>e</u> g: forage + grain production system. KH ₂ PO ₄ applied at anthesis at 10 kg material
	36	81	38	102	52	50	76	96	109	101	44	67	52	62	58	57	7	of the diffe Feekes 9 al production s anthesis at 1
	1,794	2,501	1,602	3,288	2,222	2,144	3,151	3,491	4,269	3,513	2,196	2,895	2,285	2,820	1,983	1,825	224	ED: Standard error of the difference thephon applied at Feekes 9 at 0.42. _g: forage + grain production system CH ₂ PO ₄ applied at anthesis at 10 kg 1
	1	7	ς	4	5	9	7	8	6	10	11	12	13	14	17	18	SED	SED: S Ethephic f_g: for KH ₂ PO

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NUE IN WINTER WHEAT

272													Т	НС	ЭM	AS	ON	EJ	ΓA	L.
Rate, NUE;	Chemical											Ethephon	$\rm KH_2PO_4$							
Rate, Total N	Production System	Grain	Grain Grain	Grain Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	Grain	f	f_g			Grain	Grain	Grain
Flowering N OK	Variety	2174	21/4 Jagger	Jagger	Jagger	2174	2174	Jagger	2174	2174	2174	2174	Jagger	2174	Jagger			2174	2174	Jagger
N Rate, I Tipton, C	NUE %		- 4	10	20	4	10	12	5	6		12	6	-	4	8			6-	
e, Pre-plant Iaskell, and	Total N Rate kg ha ⁻¹	0	112 0	112 63	6 9	100	96	66	101	41	78	64	61	119	120			0	112	0
opdress N Rat 1, Stillwater, F	Flowering N kg ha ⁻¹	Stillwater 0	0 0	0 (22	22	22	22	22	0	0	22	22	22	22		Haskell	0	0	0
Table 5. Treatment; Mean Grain Yield, Grain N Uptake, Topdress N Rate, Pre-plant N Rate, Flowering N Rate, Total N Rate, NUE; Variety, Production System, and Chemical Application, 2001, Stillwater, Haskell, and Tipton, OK	Winter Topdress N kg ha ⁻¹	0	0 0	0	42	78	40	43	45	41	78	42	42	41	42			0	0	0
ain Yield, Gra d Chemical A	Pre-plant N kg ha ⁻¹	0	0	112	0 0	0	34	34	34	0	0	0	0	56	56			0	112	0
nt; Mean Gr System, an	N Uptake kg ha ⁻¹	49	45 58	70 58	71	53	58	69	54	53	48	57	64	48	62	5		38	28	20
Treatment Production	Yield kg ha ⁻¹	2,061	1,744 2,677	2,721 2,723	2,710	2,201	2,153	2,892	2,222	2,054	2,039	2,224	2,447	1,809	2,303	183		1,739	1,197	1,088
Table 5 Variety,	Trt	- 0	n 1	4 v	9	٢	8	6	10	11	12	13	14	17	18	SED		1	7	б

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NUE IN WINTER WHEAT	2273
Ethephon KH2PO4	(continued)
Grain Grai Grain G	
Jagger 2174 2174 2174 2174 2174 2174 2174 Jagger Jagger 2174 2174 2174 2177 2177 2177 2177 2177	
$\begin{bmatrix} 1 & 6 \\ 5 & 3 \\ 6 & 5 \\ 6 $	
112 50 50 83 84 83 101 104 104 104 104 104 105 53 53 53 53 50 106 107 50 50 50 50 50 50 50 50 50 50 50 50 50	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
22 23 23 24 25 25 25 26 25 26 27 27 27 27 27 27 27 27 27 27 27 27 27	
976 1,627 1,627 1,626 1,626 1,626 1,535 1,535 1,491 1,491 1,488 1,488 1,488 1,488 1,491 1,594 1,4888 1,4888 1,4888 1,4888 1,488888 1,48888888888	
6 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	

		Z	Pre-plant	Winter	Flowering	Total				
	Yield		Z	Topdress N	Z	N Rate	NUE		Production	
Trt	$\rm kgha^{-1}$	kg ha ⁻¹	$\mathrm{kg}\mathrm{ha}^{-1}$	$kg ha^{-1}$	$kg ha^{-1}$	$kg ha^{-1}$	%	Variety	System	Chemical
13	3,093	64	0	29	22	51	74	2174	Grain	Ethephon
14	3,548	76	0	29	22	51	75	Jagger	Grain	$\rm KH_2PO_4$
17	3,658	86	56	47	22	125	48	2174	f_g	
18	3,824	95	56	54	22	132	44	Jagger	f B	
SED	271	8					13			

THOMASON ET AL.

Table 5. Continued

SED: Standard error of the difference between two equally replicated means. Ethephon applied at Feekes 9 at $0.42 \, \text{kg ai ha}^{-1}$.

f_g: forage + grain production system. KH₂PO₄ applied at anthesis at 10 kg material ha^{-1} in water carrier (2L).

2274

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NUE IN WINTER WHEAT

Table 6. Total Forage Yield, N Uptake and Efficiency for Forage-Only Treatments

(continued)

2276

THOMASON ET AL.

	Total	Total N. Untalia	kg Forage Yld	
Treatment	Forage Yield kg ha ⁻¹	N Uptake kg ha ⁻¹	kg N Applied	Variety
		Haskell, 2001		
15	241	5	45	2174
16	242	5	48	Jagger
17	243	6	41	2174
18	244	5	47	Jagger
		Tipton, 2001		
15	4,822	226	22	2174
16	5,208	218	24	Jagger
17	687	38	18	2174
18	1,139	59	19	Jagger

Table 6. Continued

Crop Year 1999

At the Stillwater site in 1999, we found no significant response to applied N. The block planted to 2180 experienced germination problems due to poor seed quality and could not be harvested. The Jagger plots were harvested and some high yields (3.2 Mg ha^{-1}) were noted for treatment nine (a 3-way split application with 34 kg ha⁻¹ applied pre-plant, topdress N applied based on INSEY and 22 kg N ha⁻¹ applied at flowering). The N uptake values for this plot were also indicative of good production conditions (Table 3). Forage dry matter yields and N uptake values for forage-only plots were much greater than those for forage + grain (FG) system plots (Table 7). The lack of harvest data for the 2180 plots eliminated the possibility of comparison of the two varieties for grain yield on those plots. When FG plots were compared to the grain-only plots at the same fertility and management levels, yields of grain-only plots were found to be significantly higher.

The Haskell site also experienced poor germination for the 2180 plots, thus, those plots were not harvested. Due to dry conditions in mid-spring and a very wet harvest, grain yields were highest in the 0-N check. Losses from lodging of high biomass producing plots where higher N rates were applied were significant as well as shattering losses from the heads. The greatest N uptake was from a 112 kg ha^{-1} pre-plant application (Table 3). Forage yields were greatest for the forage-only (two-cut) system (Table 6). We were unable to compare the two varieties for yield (FG treatments), but did note higher grain yields for the grain-only system when compared to the FG plots.

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i		I	I	Total	,					
2 2 3	Grain N Uptake, kg ha ⁻¹	Forage Yield, kg ha ⁻¹	Forage N Uptake, kg ha ⁻¹	N Uptake, Forage + Grain, kg ha ⁻¹	Topdress N Rate, kg ha ⁻¹	Pre-plant N Rate, kg ha ⁻¹	Flowering N Rate, kg ha ⁻¹	Total N Rate, kg ha ⁻¹	kg DM Yield/kg N Applied	Variety
				Stillwater, 1999	1999		1			
	57	951	22	79	76	56	22	154	L	Jagger
				Stillwater, 2000	2000					
	87	1,770	75	162	22	56	22	100	18	2174
	74	1,303	46	120	12	56	22	90	19	Jagger
				Stillwater, 200	2001					
	48	687	33	81	41	56	22	119	21	2174
	62	1,138	61	123	42	56	22	120	29	Jagger
				Haskell, 1999	666					
	35	412	12	47	34	56	22	112	5	Jagger
				Haskell, 2000	000					
	37	561	20	57	28	56	22	106	19	2174
	16	584	23	39	27	56	22	105	11	Jagger
				Haskell, 200	001					
	38	243	9	4	26	56	22	104	17	2174
	24	244	5	29	28	56	22	106	11	Jagger

NUE IN WINTER WHEAT

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2278

THOMASON ET AL.

	Flowering Total kg DM N Rate, N Rate, Yield/kg kg ha ⁻¹ kg ha ⁻¹ N Applied Variety	22 159 4 2174		88 24	22 82 26 Jagger		22 125 35 2174	132
	Pre-plant N Rate, kg ha ⁻¹	56	56	56	56		56	56
ıtinued	Topdress N Rate, kg ha ⁻¹	99 81	105	2000 10	4	01	47	54
Table 7. Continued	Total N Uptake, Forage + Grain, kg ha ⁻¹	Tipton, 1999 97	113	л,	129 118 Tipton, 2001	124	154	
	Forage N Uptake, kg ha ⁻¹	21	57	71	64		38	59
	Forage Yield, kg ha ⁻¹	482	1,530	1,530 1,589 1,544		687	1,138	
	Grain N Uptake, kg ha ⁻¹	76	56	58	54		86	95
	Grain Yield, kg ha ⁻¹	2,568	1,774	1,983	1,825		3,658	3,824
	Treatment	17	18	17	18		17	18

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NUE IN WINTER WHEAT

The 1999 data from the Tipton experiment did include the 2180 plots so the entire experiment was harvested. Yields for the pre-plant only N application were lower than the 3-way split application system (3.8 vs. 3.0 Mg ha^{-1}) for the 2180 plots. The same treatments applied to the Jagger plots had no effect on grain yield. No significant difference in NUE was noted for either pre-plant or split applications for either variety (Table 3). The highest NUE for this site was found for the 3-way INSEY split application to Jagger (101%). This response could be due to near ideal conditions for fall growth in 1998. Wheat plants would have taken up a large amount of N early in the season and plots with sufficient N applied early would have had the opportunity to accumulate N during more favorable conditions than those experienced later in the season. Less than ideal environmental conditions occurred in the spring of 1999 at this location with warm and dry conditions favoring volatilization of topdress and flowering applications. A late-season hailstorm also damaged yields at this site. Yields for the forage-only Jagger plots were significantly higher compared to 2174 (Table 6). Again, total dry matter yields for the forage-only (two harvest) plots were higher than the yields from the combination plots. There was no difference in yield between varieties within the FG plots.

Crop Year 2000

In the 2000 crop year, due to lack of availability of quality seed, the wheat variety 2174 was substituted for 2180 at all locations. At Stillwater, the highest yields were obtained when 78 kg N ha^{-1} was applied topdress in the spring with an additional flowering application of 22 kg ha⁻¹ (Trt. 7, Table 4). The highest NUE was 59% for trt. 9, receiving N as a 3-way split with winter topdress N applied based on INSEY for a total N rate of 70 kg ha^{-1} . Nitrogen use efficiency values for the 3-way split application using INSEY adjusted topdress rates were both greater than 47%. There was no difference in forage yield for the forage only plots between varieties in 2000. The forage-only system had higher dry matter yields than the single cutting from the FG plots (Table 7). Total N uptake values for these plots were also higher than those for FG plots. Grain yields from the grain-only vs. the FG plots were not significantly different (Table 4).

At Haskell in 2000, the highest yielding treatment was 2174 with 34 kg N ha^{-1} applied pre-plant, topdress N applied based on INSEY and another 22 kg ha^{-1} N applied at flowering (Trt. 8) (Table 4). Comparison of the treatments receiving chemical applications and those otherwise treated the same revealed no differences in final yield. Grain N uptake was the highest for the 2174 plot receiving the 3-way split application of fertilizer. This plot also had the highest NUE. The fixed topdress plots also had high NUE at this location. The three-way

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THOMASON ET AL.

split application produced one of the better NUEs even for the lower yielding Jagger plots (8% for trt 9, Table 4). Grain yield for the grain-only plots were greater than yields from FG plots. Forage yields from forage-only plots with two harvests were significantly higher when compared to FG plots. There was no difference of variety using either system.

At Tipton, the Jagger plots receiving a 3-way split application gave the highest yields and NUE values (Table 4). The 2174 plots receiving no pre-plant N and 78 kg ha⁻¹ topdress out yielded plots employing INSEY N rates at this site (2895 vs. 2196 kg ha⁻¹). At the 40 kg ha⁻¹ fixed topdress rate, yields were not different than those from the INSEY plots. This seems to indicate that those treatments where N rates were based on INSEY were not high enough to maximize yields or that the plots fertilized based on the INSEY index were penalized by not having enough pre-plant N applied and thus yields were underestimated when compared to plots with pre-plant N applied. Nitrogen use efficiency was highest for the pre-plant only applications. It seems that at the Tipton site, pre-plant fertilization is necessary to produce high N use efficiency. Forage-only plots had higher dry matter yields than those from the forage + grain system. Values for N uptake were also higher for forage-only plots. Grain yields were higher for grain-only plots when compared to forage + grain treatments.

Crop Year 2001

Yields at the Stillwater site, in 2001 were greatest with Jagger and 112 kg N ha^{-1} applied pre-plant (2721 kg ha⁻¹). This same yield level was achieved when Jagger was fertilized based on INSEY and 22 kg N ha^{-1} applied at flowering for a total N rate of 64 kg ha^{-1} , 48 kg N ha^{-1} less than that required to achieve the same yield using only a pre-plant application (Table 5). Nitrogen uptake was greatest for this same treatment (71 kg N ha⁻¹). Maximum values for NUE were 14 and 20% for 2174 fertilized with variable rates based on INSEY and 22 kg N ha⁻¹ applied at flowering and Jagger fertilized based on INSEY and 22 kg N ha⁻¹ applied at flowering, respectively.

At Haskell, yields were highest for 2174 fertilized based on INSEY with no other applications (1755 kg ha⁻¹). The 0-N check was the next highest yielding at 1739 kg ha⁻¹. This indicates a minor response to applied N at this site potentially due to large amounts of available soil NO₃. Uptake of N was greatest for 2174 receiving 34 kg N ha^{-1} pre-plant, topdress N based on INSEY and 22 kg N ha^{-1} applied at flowering (Table 5). The highest NUE of 28 % was found with Jagger fertilized based on INSEY.

At Tipton, maximum yields of 3824 and 3775 kg ha^{-1} were achieved when Jagger was fertilized with 56 kg N ha^{-1} plus an INSEY based topdress rate, plus 22 kg N ha^{-1} at flowering, and when 2174 was fertilized with 34 kg N ha^{-1} and a

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NUE IN WINTER WHEAT

fixed topdress rate of 45 kg N ha^{-1} , respectively. These yields were more than double those of the 0 N check and when comparing the 3-way split (total N rate of 101 kg ha⁻¹) to 112 kg N ha^{-1} applied pre-plant, yield was increased 125 kg ha^{-1} . Jagger fertilized with a 3-way INSEY split application and 2174 with a 3-way split N application also produced maximum values for N uptake (Table 5). The highest NUE was found with an INSEY based topdress N application to 2174 (Table 5). In fact, 2174 with INSEY based 3-way split N produced an NUE of 81% compared to 45% for pre-plant N and 46% for 78 kg N ha⁻¹ as a fixed topdress rate.

CONCLUSIONS

Overall, the highest grain yields were achieved with $34 \text{ kg N} \text{ ha}^{-1}$ pre-plant, winter topdress applications based on INSEY, and 22 kg N ha^{-1} applied at flowering. Limited differences were noted between varieties. Maximum NUE values were obtained with different treatment combinations in different years. Various combinations where INSEY was used to determine N rates were always among the best, as well as Jagger with a $112 \text{ kg N} \text{ ha}^{-1}$ pre-plant application. The high average NUE for this treatment appears mainly due to the results from the Tipton site. While residual nitrate from soil tests were not lower than the other locations, the effects of pre-plant N seems to be much greater at Tipton. This may be due to the warmer temperatures and lower rainfall generally experienced in the spring, as compared to the other sites, placing more importance on early season growth and N assimilation. As expected, forage dry matter yields for the two-cut forage-only system were greater than those from the combination grain and forage plots. In addition, grain yields for the grain-only plots were higher than yields for the forage + grain plots. In some instances, like those with both forage and grain removal, large values for yield led to large values for NUE, sometimes over 100%. These results are consistent with those found by Thomason et al.^[5] where NUE values over 100% were often noted following years with low potential for utilization of N fertilizer. The advantage of this forage + grain system is to use the forage biomass for grazing without significantly damaging final grain yields, thereby increasing nutrient use efficiency. Problems with forage harvest methods and timing limited final grain yields in some instances and therefore NUEs observed may be lower than normally expected. Choosing efficient varieties and the application of low rates, or even 0 N, at planting, basing in-season topdress rates on INSEY recommendations, and applying foliar N at flowering seems to be the most efficient way to supply N when grain production is the goal.

2282

THOMASON ET AL.

REFERENCES

- Moll, R.H.; Kamprath, E.J.; Jackson, W.A. Analysis and Interpretation of Factors Which Contribute to Efficiency to Nitrogen Utilization. Agron. J. 1982, 74, 562–564.
- Eghball, B.; Maranville, J.W. Interactive Effects of Water and Nitrogen Stresses on Nitrogen Utilization Efficiency, Leaf Water Status and Yield of Corn Genotypes. Commun. Soil Sci. Plant Anal. 1991, 22, 1367–1382.
- Kanampiu, F.K.; Raun, W.R.; Johnson, G.V. Effect of Nitrogen Rate on Plant Nitrogen Loss in Winter Wheat Varieties. J. Plant Nutr. 1997, 20, 389–404.
- Cox, M.C.; Qualset, C.O.; Rains, D.W. Genetic Variation for Nitrogen Assimilation and Translocation in Wheat. II. Nitrogen Assimilation in Relation to Grain Yield and Protein. Crop Sci. 1985, 25, 435–440.
- Thomason, W.E.; Raun, W.R.; Johnson, G.V. Winter Wheat Fertilizer Nitrogen Use Efficiency in Grain and Forage Production Systems. J. Plant Nutr. 2000, 23, 1505–1516.
- O'Leary, M.J.; Rehm, G.W. Nitrogen and Sulfur Effects on the Yield and Quality of Corn Grown for Grain and Silage. J. Prod. Agric. 1990, 3, 135–140.
- Morgan, J.A.; Parton, W.J. Characteristics of Ammonia Volatilization from Spring Wheat. Crop Sci. 1989, 29, 726–731.
- Harper, L.A.; Sharpe, R.R.; Langdale, G.W.; Giddens, J.E. Nitrogen Cycling in a Wheat Crop: Soil, Plant, and Aerial Nitrogen Transport. Agron. J. 1987, 79, 965–973.
- Tsai, C.Y.; Dweikat, I.; Huber, D.M.; Warren, H.L. Interrelationship of Nitrogen Nutrition with Maize (*Zea mays*) Grain Yield, Nitrogen Use Efficiency and Grain Quality. J. Sci. Food Agric. 1992, 58, 1–8.
- Pan, W.L.; Kamprath, E.J.; Moll, R.H.; Jackson, W.A. Prolificacy in Corn: Its Effects on Nitrate and Ammonium Uptake and Utilization. Soil Sci. Soc. Am. J. **1984**, *48*, 1101–1106.
- 11. Salsac, L.; Chaillou, S.; Morot-Gaudry, J.F.; Lesaint, C.; Jolivoe, E. Nitrate and Ammonium Nutrition in Plants. Plant Physiol. Biochem. **1987**, *25*, 805–812.
- 12. Ellen, J.; Spiertz, J.H.J. Effects of Rate and Timing of Nitrogen Dressings on Grain Yield Formation of Winter Wheat. Fert. Res. **1980**, *1*, 177–190.
- Olson, R.V.; Swallow, C.W. Fate of Labeled Nitrogen Fertilizer Applied to Winter Wheat for Five Years. Soil Sci. Soc. Am. J. 1984, 48, 583–586.
- Wuest, S.B.; Cassman, K.G. Fertilizer-Nitrogen Use Efficiency of Irrigated Wheat. I. Uptake Efficiency of Preplant vs. Late-Season Application. Agron. J. 1992, 84, 682–688.

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NUE IN WINTER WHEAT

- 15. Wuest, S.B.; Cassman, K.G. Fertilizer-Nitrogen Use Efficiency of Irrigated Wheat. II. Partitioning Efficiency of Preplant vs. Late-Season Application. Agron. J. **1992**, *84*, 689–694.
- Raun, W.R.; Solie, J.B.; Johnson, G.V.; Stone, M.L.; Whitney, R.W.; Lees, H.L.; Sembiring, H.; Phillips, S.B. Micro-Variability in Soil Test, Plant Nutrient and Yield Parameters in Bermudagrass. Soil Sci. Soc. Am. J. 1998, 62, 683–690.
- 17. Solie, J.B.; Raun, W.R.; Whitney, R.W.; Stone, M.L.; Ringer, J.D. Optical Sensor Based Field Element Size and Sensing Strategy for Nitrogen Application. Trans. ASAE **1996**, *39* (6), 1983–1992.
- Solie, J.B.; Raun, W.R.; Stone, M.L. Submeter Spatial Variability of Selected Soil and Bermudagrass Production Variables. Soil Sci. Soc. Am. J. 1999, 63, 1724–1733.
- Stone, M.L.; Solie, J.B.; Raun, W.R.; Whitney, R.W.; Taylor, S.L.; Ringer, J.D. Use of Spectral Radiance for Correcting In-Season Fertilizer Nitrogen Deficiencies in Winter Wheat. Trans. ASAE 1996, 39, 1623–1631.
- 20. Van Sanford, D.A.; Grove, J.H.; Grabau, L.J.; MacKown, C.T. Ethephon and Nitrogen Use in Winter Wheat. Agron J. **1989**, *81*, 951–954.
- 21. Benbella, M.; Paulson, G.M. Efficacy of Treatments for Delaying Senescene of Wheat Leaves. II Senescene and Grain Yield Under Field Conditions. Agron. J. **1988**, *90*, 332–338.
- 22. Lukina, E.V.; Freeman, K.W.; Wynn, K.J.; Thomason, W.E.; Mullen, R.W.; Klatt, A.R.; Johnson, G.V.; Elliott, R.L.; Stone, M.L.; Raun, W.R. Nitrogen Fertilization Optimization Algorithm Based in In-Season Estimates of Yield and Plant Nitrogen Uptake. J. Plant Nutr. **2001**, *24*, 885–898.
- Raun, W.R.; Solie, J.B.; Johnson, G.V.; Stone, M.L.; Lukina, E.V.; Thomason, W.E.; Schepers, J.S. In-Season Prediction of Potential Grain Yield in Winter Wheat Using Canopy Reflectance. Agron. J. 2000, 93, 131–138.
- 24. Large, E.C. Growth Stages in Cereals. Plant Path. 1954, 3, 128–129.
- Schepers, J.S.; Francis, D.D.; Thompson, M.T. Simultaneous Determination of Total C Total N and 15N on Soil and Plant Material. Commun. Soil Sci. Plant Anal. **1989**, *20* (9&10), 949–959.
- SAS Institute. SAS/STAT User's Guide, Version 6, 4th Ed.; SAS Inst.: Cary, NC, 1989; Vol. 2.