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The Effects of Stabilized Urea and Split-Applied Nitrogen on Sunflower Yield and Oil Content

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Abstract

Sunflower is an efficient nitrogen (N) accumulator due to its aggressive taproot and extensive root system. While N rate studies in sunflower have shown a yield response, the response is often highly variable and difficult to predict in many instances. Additionally, since most sunflower production is intended for the oil market, surplus nitrogen tends to decrease oil content. Therefore, it is critical to hone nitrogen rates to maximize both yield and oil production and to incorporate alternative approaches to fertilizer application, which includes timing and method of application. The objective of the present study was to assess the efficacy of a split-application of N at either the V4 or R1 growth stage to increase yield and/or oil content in sunflower. A second objective was to examine whether a urease inhibitor could be used to retain soil N longer and achieve a similar effect as a split-application. Studies were conducted at two locations over two growing seasons in South Dakota, USA. A target rate of 90 kg·ha^{-1} was applied as urea-ammonium nitrate (UAN) either as an at-planting application or split-applied. Overall, N additions did significantly increase yield over a control. On average, the urease inhibitor tended to increase grain yields over split-applying N at either growth stage, however, there was no statistical effect on either grain yield or oil content. Based on ^{15}N analysis, approximately 27% of the N in the grain was derived from the UAN fertilizer, which indicates a relatively large reliance upon soil N for grain N content. The addition of a urease inhibitor significantly increased average fertilizer uptake by nearly 6% to 32.7%.

Keywords

Nitrogen Uptake, Urease Inhibitor, Isotopic Nitrogen, Nitrogen Use Efficiency

1. Introduction

Relative to other plants, sunflower (*Helianthus annuus* L.) utilizes N in a fairly efficient manner [1]. Sunflower is known to have an aggressive taproot, reaching maximum rooting depths of 1 - 3 m by the grain-filling period [2]. Root density, however, tends to decrease exponentially with depth with up to ten times greater root mass in the 0 - 0.2 m soil layer compared to deeper soil depths [3]. Rooting depth typically correlates with water extraction with maximum depths reached in wetter growing seasons [4]. Likewise, the majority of N is taken up by the plant through mass flow. Thus, N uptake dynamics often mimics water uptake patterns with more N being taken up during wetter years and less during periods of drought.

Sunflower grain yield has long been known to respond to fertilizer N application, particularly at extractable available soil N levels less than 60 kg·ha^{-1} [5]. However, because of the rooting dynamics of sunflower, it is often difficult to predict the extent of response to N fertilizer. For example, researchers in North Dakota detected a significant yield increase from N application in just two out of nine years [6]. Additionally, as fertilizer N rate increases, grain oil content of sunflower tends to decrease [7].

Beyond application rate, timing of N application is another important aspect of an effective fertilization program and much less is known in regards to N uptake by sunflower. Previous research indicates that seed weight can be increased by fertilizer N application at various stages of the growing season, but may be most affected when fertilization is timed between floret initiation and anthesis [8]. Furthermore, Goswami and Srivastava [9] noted that sunflower roots continue to absorb soil N even into the grain filling period, suggesting that later N application may also increase grain yield.

Given the nature of sunflower rooting dynamics, the likelihood of excess N post-harvest is high. Indeed, Schatz *et al.* [6] indicated an average of approximately 33 kg·ha^{-1} of residual soil nitrate-N at an application rate 90 kg N ha^{-1}. As a result, over-application of fertilizer N to sunflower could have many deleterious effects. Excess N in agricultural systems has been linked to numerous environmental problems including increased hypoxic zones in coastal areas, contaminated drinking water, decreased biodiversity and increased global warming effects [10] [11] [12] [13].

In order to maximize fertilizer N use efficiency (FNUE), it is important to determine when fertilizer N application is most effective and environmentally beneficial. The objectives of this study were to determine FNUE, grain yield, and oil content in sunflower as affected by timing of fertilizer N application (at-planting, V4, R1 growth stages) or through the use of a urease-inhibitor. Both options have been shown to increase N use efficiency, maintain or increase yields and minimize environmental impacts [14] [15]. However, other researchers have found mixed results [16] [17]. Moreover, much of the research on this topic has focused on maize and small grains. There is a dearth of information with respect to these approaches on sunflower, a crop with a very different rooting system.

2. Materials and Methods

2.1. Study Site Characteristics and Study Design

This study took place at Bison (45˚30'N, 102˚33'W) and Onida, SD, USA (44˚42'N, 100˚15'W) in 2014 and was reduced to just the Onida site (44˚35'N, 100˚05'W) in 2015. This research was conducted on-farm with the Onida site separated by approximately 24 km between years. Selected soil characteristics by site at the initiation of this research are listed in Table 1. The experiment was arranged in a complete randomized block design with four replications for each treatment. The main effect consisted of five N treatments using a target application rate of 90 kg·ha^{-1}. The N treatments were as follows: Control (0N), 90 kg N ha^{-1} applied at planting (90AP), 90 kg N ha^{-1} with *N-(n-butyl)-thiophosphoric triamide* (NBPT) urease inhibitor (90AP + NBPT), 90 kg N ha^{-1} split-applied with 50% at planting and 50% applied at the V4 (four true leaves at least 4 cm in length) growth stage (90 SplitV4) and 90 kg N ha^{-1} split-applied with 50% at planting and 50% applied at the R1 (terminal bud formation) growth stage (90 SplitR1). All fertilizer N was banded at planting as a urea-ammonium-nitrate solution (UAN, 28-0-0). The split-application was dribbled approximately 7 cm off each row and applied by hand. The determination of growth stages were evaluated using the stages developed by Schneiter and Miller [18].

Sunflower (Mycogen Seeds MY8H456CL, Size 3, Indianapolis, IN) was planted with a no-till grain drill (Model 750, John Deere Co., Moline, IL) at a population of 4.1 plants m^{-2} on 9 and 12 June, 2014 for Bison and Onida, respectively. In 2015, the Onida site was planted on 10 June. Thiamethoxam (*3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-N-nitro-1,3,5-oxadiazinan-4-imine*) (Syngenta, Wilmington, DE) was applied as an insecticide seed treatment at a rate of 0.25 mg a.i. per seed. Each plot consisted of four rows planted at 76.2 cm

Table 1. Summary of soil attributes at each study site.

Location	Bison	Onida	
		2014	2015
Soil Texture	Sandy Clay Loam	Silty Clay Loam	Silty Clay Loam
Sand (0 - 15 cm, g·kg^{-1})	550	190	190
Silt (0 - 15 cm, g·kg^{-1})	200	420	470
Clay (0 - 15 cm, g·kg^{-1})	250	390	340
pH (1:1 water)	5.9	6.0	6.5
Organic Matter (0 - 15 cm, g·kg^{-1})	16	31	42
P (0 - 15 cm, mg·kg^{-1})	21	10	23
N (0 - 15 cm, kg·ha^{-1})	4	31	10
N (15 - 60 cm, kg·ha^{-1})	12	65	54
K (0 - 15 cm, mg·kg^{-1})	464	558	475
Soluble Salts (mmho cm^{-1})	0.3	0.5	0.4

between rows and 9.1 m long. For weed control, Sulfentrazone (*N-{2,4-Dichloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-yl]phe nyl}methanesulfonamide*) (FMC, Philadelphia, PA) and glyphosate (*N-[phosphonomethyl] glycine*) were applied at planting for each site.

2.2. Isotopic N Analysis

Fertilizer recovery was evaluated using ^{15}N-labeled UAN (1.366‰ ^{15}N atom excess) applied in a micro-plot (3.9 m^2) established within the center of each plot. This plot size was assumed to be sufficient to eliminate border effects based on research from other crops [19]. Labeled N was applied by hand to mimic application in the larger plot to ensure compatibility. At physiological maturity, four plants were hand-harvested at the soil surface from the center of each microplot. Due to an unusually early frost (September 8, 2014) accurate biomass (lamina) samples were only obtained from the Onida location in 2015. The plants were separated into component parts (grain, head, stalk and leaves), dried at 70˚C for 72 hours, weighed, and ground to pass a 0.5 mm sieve. Nitrogen content and ^{15}N atom excess were determined using an NC1500 (Carlo Erba, Milan, Italy) automated dry combustion analyzer coupled to an Isoprime (Micromass, Beverly, MA) mass spectrometer. All prepared samples were run in duplicate. The fraction of N derived from fertilizer (Ndff) was calculated as:

$$F = \frac{(A_s - A_r)}{(A_f - A_r)}$$

and

$$E_f = F \times N_u$$

where F is the fraction of total N uptake derived from ^{15}N enriched fertilizer, A_s is the atom % ^{15}N measured in the harvested plant sample, A_f is the atom % ^{15}N in the enriched fertilizer, A_r is atom % ^{15}N of the reference harvested plant material from non-^{15}N enriched control plots and E_f is the total uptake of ^{15}N enriched fertilizer and N_u is the total N uptake by the plant or plant component [20].

2.3. Statistical Procedures

The study was arranged in a 5 × 2 × 2 split-split-block factorial arrangement with the N treatment randomly applied within each of four replications. Grain yield, oil content, N uptake and residual N were analyzed statistically as a linear mixed-effects ANOVA model with Satterthwaite's approximation for denominator degrees of freedom using the lme4( ) and lmerTest( ) modules [4] [21] in R statistical package [1]. All of the data were obtained from the harvested microplots and results are presented at this scale. Nitrogen treatment, a categorical factor with 5 levels and Location, a categorical factor with 2 levels, were analyzed as fixed effects. To account for the high variability between the years of the study and difference in the number locations, Year was analyzed as a random effect, which assumes a different baseline for measured indicators based on each year. Interactions were assessed statistically for each combination of the fixed effects. Levene's test was used to check for homogeneity of variance. Residual and Q-Q plots were applied to examine data normality. Further assumptions of the linear package were verified using the gvlma( ) based on Pena and Slate [22]. Significance was determined at $P \leq 0.05$ (unless otherwise stated) with means separation determined by Tukey's Honest Significant Difference method. All linear relationships were analyzed with the lm( ) function using the R statistical package.

3. Results and Discussion

3.1. Grain and Oil Yield and N Uptake

In general, the timing of N supply did not have a significant effect on grain yield, N uptake or oil yield. A single application at planting was as effective as a split-application. Hocking and Steer [23] noted that maximum N uptake in sunflower is the period between floret initiation and anthesis, which implies a necessity for adequate N supply just prior to floret initiation to ensure maximum yield. In 2014 in particular, planting was influenced by above average rainfall and cooler average temperatures (Table 2). This likely impacted seed germination, emergence, and stand uniformity. Overall, grain yield was significantly increased by N treatments. Across all treatments, average grain yield was increased by approximately 65%. Within N treatments, however, grain yield was not statistically different (Table 3). Similar inconsistent results have been recorded in maize

Table 2. Temperature and precipitation for the sites and years in the study and the 30-year average for each site.

		Bison (2014)	Bison 30-Yr Average	Onida (2014)	Onida (2015)	Onida 30-Yr Average
Temperature (C)	April	5.3	7.3	6.6	9.4	7.7
	May	12.8	13.4	13.7	12.4	13.9
	June	15.6	18.4	17.6	19.9	19.4
	July	20.4	22.6	20.8	23.4	23.3
	Aug	21.1	22.1	20.8	21.7	22.2
	Sept	15.5	16.1	16.8	19.1	16.7
	Oct	10.5	8.7	9.8	11.2	8.3
	Average	14.5	15.5	15.2	16.7	16.0
Precipitation (mm)	April	44.7	46.7	59.4	11.7	47.5
	May	38.4	78.5	63.5	138.9	77.7
	June	205.0	73.9	136.4	84.1	84.8
	July	22.1	60.2	23.6	34.3	67.3
	Aug	82.8	41.1	67.1	70.9	59.9
	Sept	46.2	32.3	19.1	48.8	45.7
	Oct	7.6	37.3	15.0	34.3	42.4
	Total	446.8	370.1	384.0	422.9	425.5

Table 3. Main effects of N Treatment and Location on yield and N uptake indicators.

Main Effect/Level†	Yield		Percent N in Grain		N Uptake		Yield: N Ratio	
	g plot^{-1}		%		g plot^{-1}			
N Treatment								
Control	84.1	a	2.95	a	2.08	a	35.62	a
90 AP	139.9	b	3.23	a	4.56	b	31.66	b
90 AP + NBPT	147.2	b	3.09	a	4.42	b	32.87	b
90 Split V4	129.9	b	3.04	a	3.82	b	33.43	ab
90 Split R1	145.9	b	3.23	a	4.67	b	32.05	b
Location								
Bison	66.0	a	3.39	a	2.35	a	29.95	a
Onida	192.9	b	2.89	b	5.47	b	36.31	b
ANOVA								
N Treatment	*		NS		***		*	
Location	***		***		***		***	
N Treatment*Location	NS		*		NS		**	

*Statistical significance at 0.05; **Statistical significance at 0.01; ***Statistical significance at 0.001; NS, not significant. †Effects are compared within each column and main effect. Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

from numerous possible contributing factors including soil type, weather, rotation and application method [24]. Likewise, N uptake was increased in a similar manner for all N additions; again with no statistical differences among N timing treatments. Moreover, percent N in grain followed a similar trend, but the addition of N did not significantly increase N concentrations in any of the treatments (**Table 3**).

Oil content did not differ by N treatment but did vary significantly by location with Bison averaging 36.3% and Onida averaging 44.6% (**Table 4**). Moreover, the yearly effect (data not shown) was significant ($P < 0.001$) at Onida with oil content averaging 41.1% in 2014 and 47.7% in 2015. However, for oil yield (oil content x grain yield) both N treatment ($P = 0.02$) and Location ($P < 0.001$) were statistically significant effects, following a similar pattern as overall grain yield trends. **Figure 1** shows the large disparity in oil yield between the two study sites. Due to the heavy rainfall early in the growing season in 2014, oil production was extremely low, particularly at the Bison site.

In general, applying fertilizer N increased oil yield. Because oil content was not materially different between treatments, this effect was largely due to the increased grain yield. Contrary to our initial hypothesis, a later N application did not increase either oil content or yield. The addition of a urease inhibitor was as effective as a split-application and in fact produced the highest oil yield on aver-

Table 4. Main effects of N Treatment and Location on yield and N uptake indicators.

Main Effect/Level	Oil Content	
	%	
<u>N Treatment</u>		
Control	40.9	a
90 AP	41.0	a
90 AP + NBPT	40.5	a
90 Split V4	39.9	a
90 Split R1	39.9	a
<u>Location</u>		
Bison	36.3	
Onida	44.6	
ANOVA		
N Treatment	NS	
Location	***	
N Treatment*Location	NS	

†Effects are compared within each column and main effect. Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

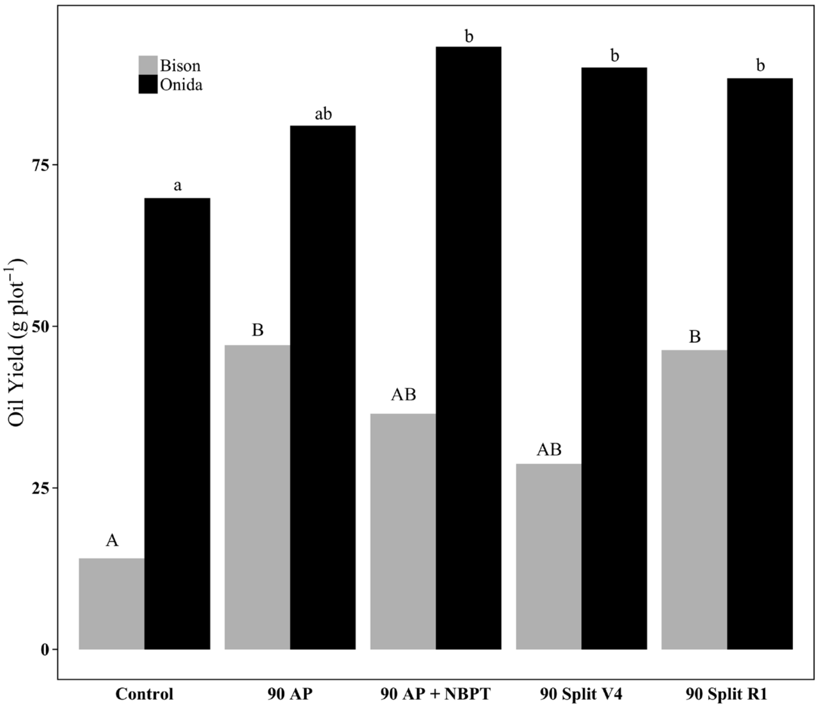

Figure 1. Oil yield (g plot^{-1}) by study site. Treatments consist of Control (0N), 90 kg N ha^{-1} applied at planting (90 AP), 90 kg N ha^{-1} with urease inhibitor (90AP + NBPT), 90 kg N ha^{-1} split-applied with 50% at planting and 50% applied at the V4 growth stage (90 Split V4) and 90 kg N ha^{-1} split-applied with 50% at planting and 50% applied at the R1 growth stage (90 Split R1). Effects are compared within each site. Mean values followed by the same letter (and case) are not significantly different at $P \leq 0.05$.

age. However, this difference was not statistically different than other N addition methods.

3.2. N Derived from Fertilizer in Plant Components

Based on ^{15}N analysis, approximately 27% of the N in the grain was derived from the UAN fertilizer, which indicates a relatively large reliance upon soil N for final grain N content. The addition of a urease inhibitor significantly increased average fertilizer uptake by nearly 6% to 32.7%. When compared to the standard practice of applying UAN at planting, the urease inhibitor showed a trend toward increased yield as well, which improved the overall efficiency of the fertilizer.

Moreover, the sources for the final N concentration in the grain are a mix of N derived directly from the soil and N mobilized by the photosynthetic apparatus, with little contribution from the stalk [23]. Early studies determined that uptake of N versus mobilization of N to the sunflower grain varied from 57% and 43%, respectively, for sunflower plants with high N supply to 25% and 75%, respectively, for sunflower plants with low N supply [23] [25].

Excepting grain, location was not a significant treatment effect for Ndff in any of the analyzed plant components (**Table 5**). This result suggests that fertilizer N uptake to the other plant components is relatively stable over broad environ

Table 5. Nitrogen derived from fertilizer (Ndff) as a percentage of total N uptake for each plant component.

Main Effect/Level†	Grain Ndff		Leaf Ndff		Stalk Ndff		Head Ndff	
	%		%		%		%	
N Treatment								
90 AP	26.82	a	36.46	a	29.38	ab	30.97	ab
90 AP + NBPT	32.74	b	39.57	a	32.53	b	33.49	b
90 Split V4	27.15	a	31.66	b	28.04	a	29.59	a
90 Split R1	26.94	a	26.75	c	26.38	a	27.38	a
Average	28.41		33.61		29.08		30.36	
Location								
Bison	25.80	a	33.33	a	29.00	a	29.98	a
Onida	30.88	b	33.89	a	29.16	a	30.73	a
ANOVA								
N Treatment	*		***		.		*	
Location	*		NS		NS		NS	
N Treatment*Location	NS		NS		NS		NS	

Statistical significance at 0.10; *Statistical significance at 0.05; **Statistical significance at 0.01; ***Statistical significance at 0.001; NS, not significant. †Effects are compared within each column and main effect. Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

mental conditions. However, N derived from fertilizer, as a percent of total N in the component part, varied significantly by application timing (**Table 5**). In general, as N was applied later in the season, the concentration of fertilizer N in each plant component decreased. For example, a split-application significantly reduced the Ndff in the leaves as would be expected based on the timing of the N supply and the growth stage of the plant. Conversely, the addition of a urease inhibitor at planting increased the proportion of fertilizer N in all plant components by 3.7% - 7.7% on average over the other N treatments.

3.3. Effect of Timing and Weather on N Use

In theory, split-applying N provides a benefit over an at-planting application because the plant can better compete with early season environmental losses (*i.e.* leaching, denitrification, etc.) [8]. Based on the lack of yield response in the current results, it appears that the target N application rate (90 kg·ha^{-1}) was sufficient to maintain yield despite any early season losses.

This is likely due to a substantial reliance upon soil N for the plant's needs. When averaged across all treatments, fertilizer met only 30% of any sunflower component's needs. By applying half of the total N fertilizer at planting in the split-application treatments, the plant appears to simply shift its N source to soil reserves. However, this appears to come at the expense of relying more on soil N for its needs. Therefore, the resulting yields are similar between treatments, but they are achieved through different N sources. This dynamic likely played a large role in the location differences. Bison had much lower starting soil N and low organic matter content leaving this site with much fewer N reserves for the plant to draw upon (**Table 1**). However, these results must be regarded carefully. A full accounting of N use efficiency must factor in total N uptake, which requires measurement of total biomass. As mentioned previously, due to an uncharacteristically early frost in 2014, a significant portion of the leaf matter was sloughed off and thus unaccounted for.

However, as shown in **Figure 2**, there is a strong linear correlation between grain yield and Ndff. Moreover, the correlation between individual plant component Ndff is similarly robust (**Figure 3**). This suggests that as grain yield or biomass increases, so too does fertilizer N uptake and hence, FNUE. Based on 2015 data at Onida, the average whole-plant FNUE was 57% (unpublished). In comparison, Scheiner *et al.* [7] found an overall FNUE of 51% with a target N application rate of 75 kg·ha^{-1}.

In the drier climate at Onida, the Ndff from the 90AP + NBPT treatment did not differ from the 90AP treatment but was significantly greater than either split-application. Conversely, at Bison where rainfall was high early in the growing season, the Ndff from the 90AP + NBPT treatment was significantly greater than the 90AP, but not significantly different than the split-applications (**Figure 4**). These results suggest that there was likely some increased environmental N losses through increased rainfall and that the urease inhibitor likely protected the UAN against these conditions by reducing the conversion to NO_3-N

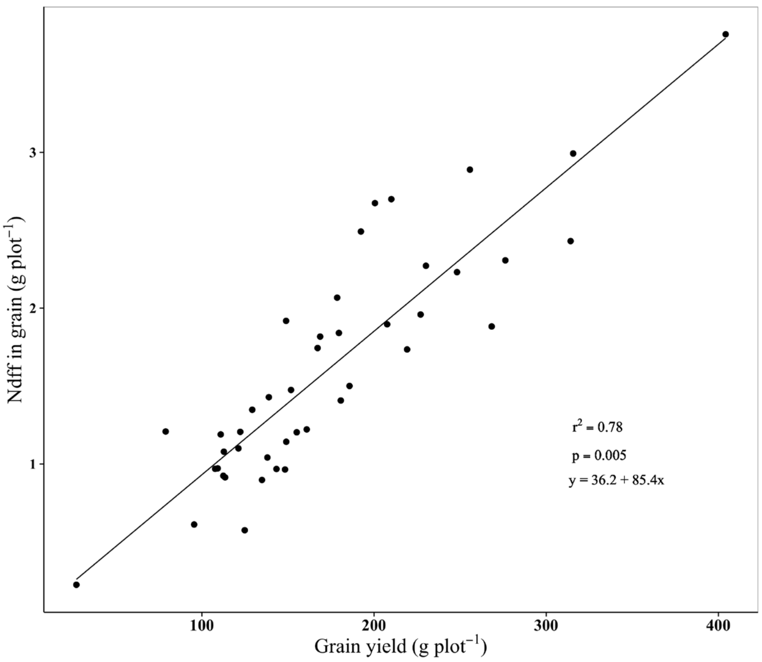

Figure 2. Relationship between sunflower grain yield and N derived from fertilizer (Ndff) in the grain at harvest.

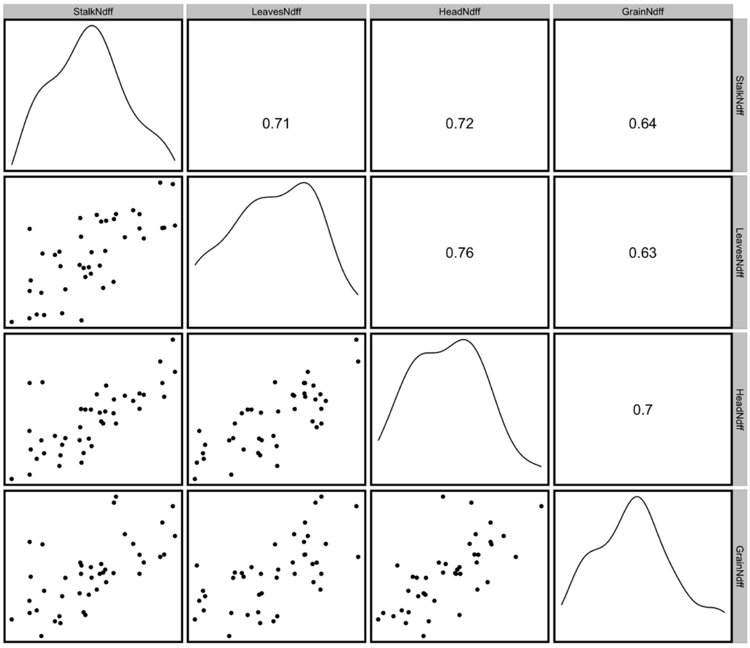

Figure 3. Pearson correlation coefficient matrix demonstrating the linear relationship between the N derived from fertilizer (Ndff) for each component part of the sunflower plant averaged across all site-years.

throughout the early growing season, which allowed for greater fertilizer N uptake. However, due to a lack of data with respect to split-application and slow-release fertilizers on sunflower, these results should be verified through additional trial replications across a broader environmental gradient.

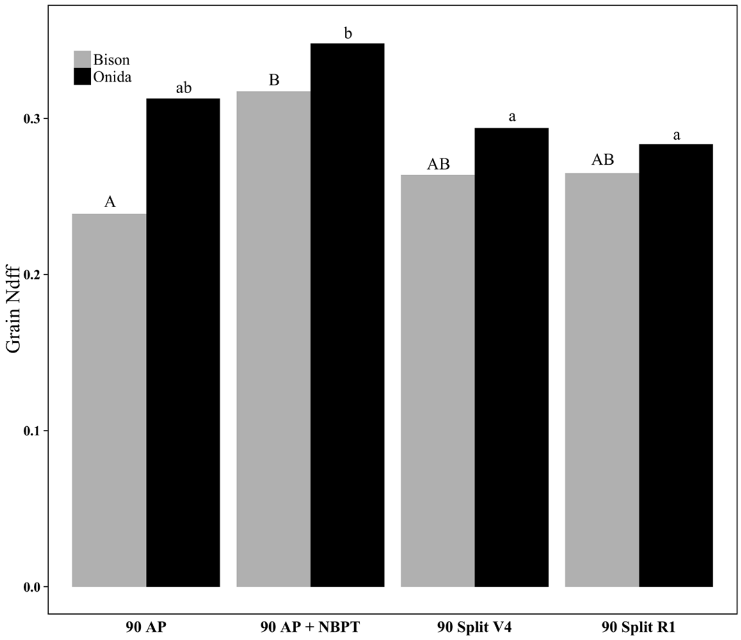

Figure 4. Nitrogen content in the grain derived from fertilizer by study site. Treatments consist of Control (0N), 90 kg N ha^{-1} applied at planting (90 AP), 90 kg N ha^{-1} with urease inhibitor (90AP + NBPT), 90 kg N ha^{-1} split-applied with 50% at planting and 50% applied at the V4 growth stage (90 Split V4) and 90 kg N ha^{-1} split-applied with 50% at planting and 50% applied at the R1 growth stage (90 Split R1). Effects are compared within each site. Mean values followed by the same letter (and case) are not significantly different at $P \leq 0.05$.

4. Conclusions

In general, these data suggest that split-applying N is as effective as an at-planting N application at increasing sunflower grain yield. Based on ^{15}N uptake, lower Ndff in component parts in the split-application treatments suggests that the plant simply shifts its reliance on fertilizer N to soil N depending upon availability.

Meanwhile, the use of a urease inhibitor with UAN does appear to increase fertilizer N uptake. There was a trend towards higher yields with the urease inhibitor; however this was not statistically significant due to high variability. By slowing down the N transformations from urea to NO_3^-, the urease inhibitor potentially reduced assumed environmental losses through increased plant uptake. The addition of a urease inhibitor significantly increased average fertilizer uptake by nearly 6% to 32.7%. This result suggests that a urease inhibitor may be an effective means for environmentally sensitive sunflower production while reducing the need for additional in-season fertilizer application.

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