VOLATILIZATION LOSSES FROM UREA

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Nitrogen fertilizer in the form of urea is subject to ammonia volatilization through the activity of the urease enzyme found ubiquitously in soil (Kissel et al., 2008). Nitrogen volatilization is especially prevalent when urea is applied to the soil surface, as in no-till systems when growers have not invested in sub-surface application tools. To decrease possible ammonia volatilization losses a number of products have been developed to delay urease activity.

Urease Inhibitors

The compound that has most consistently decreased urea volatilization when mixed with urea or urea-ammonium nitrate solutions is NBPT (N-(n-butyl) thiophosphoric acid triamide). NBPT is marketed as Agrotain® (Agrotain International LLC). The mechanism for NBPT is to lock onto the urease enzyme binding sites, preventing the enzyme from reacting to the urease (Manunza et al., 1999).

Agrotain (NBPT) decreases the rate of ammonia volatilization from urea applied to the surface as dry urea or urea-ammonium nitrate solutions (Brouder, 1996; Table 1). Ammonia volatilization losses from urea at Brandon, MB decreased from 40 mg to 2 mg and from 88 mg to 12 mg with Agrotain in two separate studies for a 7-day period after application (Grant, 2004).

In a recent Kansas study (Weber and Mengel, 2009), urea was applied in three site years to the soil surface after corn emergence using a number of nitrogen extending additives including Agrotain. The Agrotain treatment was superior to urea alone by 25 bushels per acre in one of the three site years. The two locations that received significant rainfall immediately following applications did not receive a yield benefit from the Agrotain treatment. In sorghum, urea + Agrotain and urea + SuperU were 11 and 12 bushels per acre respectively greater in yield than urea broadcast alone (Weber et al., 2009a). At two drier locations there were no yield differences between urea + Agrotain and urea alone.

A 14-year study in southern Illinois (Ebelhar et al., 2010) showed a 3 bushel corn yield advantage of urea + Agrotain compared to urea broadcast in conventional till surface and incorporated over 12 years of treatments. In no-till, urea+ Agrotain held an 11 bushel/acre advantage over urea surface applied over 4 years of treatments. Another study in southern Illinois (Varsa et al., 1999), Agrotain treated UAN surface dribbled was superior to UAN surface dribble alone (Table 2).

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Table 1. Mean corn yield from Purdue Agronomy Farm, SEPAC, Pinney Purdue and Kosciusko locations with urea and UAN alone and treated with NBPT (Brouder, 1996, citing work by Phillips, Mengel and Walker, 1989, unpublished work, Purdue, University).

Fertilizer treatment	Yield, bu/acre
Control- (20 lb N/acre in starter only	99
Urea broadcast, surface	130
Urea + NBPT broadcast, surface	143
UAN broadcast, surface	135
UAN + NBPT broadcast, surface	140
UAN dribbled, surface	139
UAN spoke injected	142
UAN coulter injected	147
UAN knife injected	145

Table 2. No-till corn yield as affected by N fertilizer sources, Agrotain and placement in Illinois (fFrom Varsa et al., 1999).

	Belleville		Dixon Springs	
Treatment	Yield, bu/acre			
Control (0N)	34	53	62	73
Urea	106	120	98	100
Urea + Agrotain	134	143	112	112
UAN, surface	123	137	103	107
UAN + Agrotain, surface	128	145	107	114
UAN, dribble	139	137	108	112
UAN + Agrotain, dribble	143	152	110	120
UAN injected	172	176	123	121
Anhydrous ammonia	158	166	122	130

In Kentucky, 50 lb N/acre was applied preplant to all corn plots (Schwab and Murdock, 2009). Side-dress applications of urea and UAN with several additives or formulations were applied to the soil surface at 6-leaf stage. Higher yields than urea alone were achieved with urea + Agrotain and SuperU. Higher yields than UAN alone were achieved with UAN + Agrotain and UAN + Agrotain Plus (combination of NBPT and DCD formulated for use with UAN) (Table 3).

Table 3. Yield for side-dressed no-till corn in Hardin County, KY (from Schwab and Murdock, 2010).

Treatment	Yield, bu/acre
Check (50 lb N/acre preplant N only)	117d*
Urea	158c
Urea + Agrotain	201b
SuperU	201b
UAN	150c
UAN + Agrotain	179bc
UAN + Agrotain Plus	175bc
Ammonium nitrate	239a

* Numbers followed by the same letter are not significantly different (5%)

Additional Possible Urease Inhibitors

Ammonium thiosulfate (ATS) and several additional commercial thiosulfates have soil urease inhibiting properties (Goos, 1985). In the process of identification of thiosulfates as soil urease inhibitors, it was noted that the compounds would not be expected to perform as well as some other alternative nitrification and urease inhibitors due to the shorter decomposition period for ATS compared to nitrapyrin (Goos, 1985). One study was unable to duplicate urease inhibition results, but used different methods than originally presented at rates of ATS from 3.3 to 33 times the rates of Goos, 1985 (McCarty et al., 1990). Thiosulfate activity is regulated by its concentration (effective at S rates of 25 mg kg^{-1} (Goos and Johnson, 2001).

Thiosulfate readily breaks down rapidly in temperatures of 15^{o}C. In a laboratory study at 15^{o}C, ATS was essentially mineralized in about a week. Under cooler temperatures; however, significant thiosulfate remained after 2 weeks in two of three soils, with mineralization complete in all soils by week 3. Cautions were expressed by Janzen and Bettany (1986) on high rates of banded ATS (over 100 ppm) due to nitrite accumulation from ATS inhibition of not only the ammonium to nitrite process, but the nitrite to nitrate process. The rate used by Goos (1985) was about 43 ppm if expressed as a band with radius 2 inches, which did not accumulate nitrite in the Janzen Bettany (1986) study. Recently, the use of thiosulfate has been reexamined. In Kansas, the application in the spring of a 5% and 10% calcium thiosulfate by volume solution with UAN had similar yield as urea broadcast in no-till (Tucker and Mengel, 2007).

Nutrisphere-N is a product marketed by SFP (Specialty Fertilizer Products) LLC, Leawood, KS. The formulation for dry fertilizer is a 30 to 60% maleic itaconic co-polymer calcium salt. The pH of the dry formulation is between 2.5 and 5 according to the label. The rate of use is 0.5 gallon per ton of urea/ammonium sulfate. The formulation for liquid fertilizer is a 40% minimum maleic-itaconic co-polymer. The pH of the liquid product is between 1 and 2 according to the label. The rate of mixing with liquid N products is 0.5 gallon Nutrisphere-N per 99.5 gallons of fertilizer solution. A gallon of Nutrisphere-N liquid or dry formulation weighs 9.6 pounds per gallon. Nutrisphere-N is marketed as both a urease inhibitor and a nitrification inhibitor. The activity of the product on urease is theoretically based on its binding to nickel ions necessary for the formation and function of

the enzyme. The most consistent yield increases and crop uptake of N from the use of Nutrisphere-N has been through work by Gordon (2008). In 2 years of corn at Scandia, KS and 2 years of grain sorghum at Belleville, KS, yield increases to the use of Nutrisphere-N were similar to those achieved with urea-Agrotain and ESN (Tables 4 and 5).

Table 4. Effects of N additive, averaged over source (UAN and urea) and N rate on corn grain yield, earleaf-N and grain-N, Scandia, KS (2-year average) (from Gordon, 2008).

Treatment	Yield, bu/acre	Earleaf N, %	Grain N, %
Check	152	1.72	1.13
Urea/UAN	168	2.57	1.26
ESN	185	2.96	1.33
Nutrisphere-N	183	2.96	1.35
Agrotain	183	2.98	1.36
LSD 5%	6	0.09	0.04

Table 5. Effects of N source and rate on grain sorghum yield, Belleville (2-year average). (from Gordon, 2008).

Treatment	N-Rate, lb/acre	Yield, bu/acre
Check	0	71
Urea	40	108
	80	122
	120	128
ESN	40	120
	80	130
	120	132
Urea + Agrotain	40	116
	80	129
	120	133
Urea+ Nutrisphere	40	120
	80	133
	120	132
LSD 5%		5

The consistent results from Gordon (2008) are very curious considering that careful laboratory experiments by Goos (2008) and Norman (Franzen et al., 2011) have shown that Nutrisphere-N has no urease inhibitor ability (Fig. 1-4, Table 6).

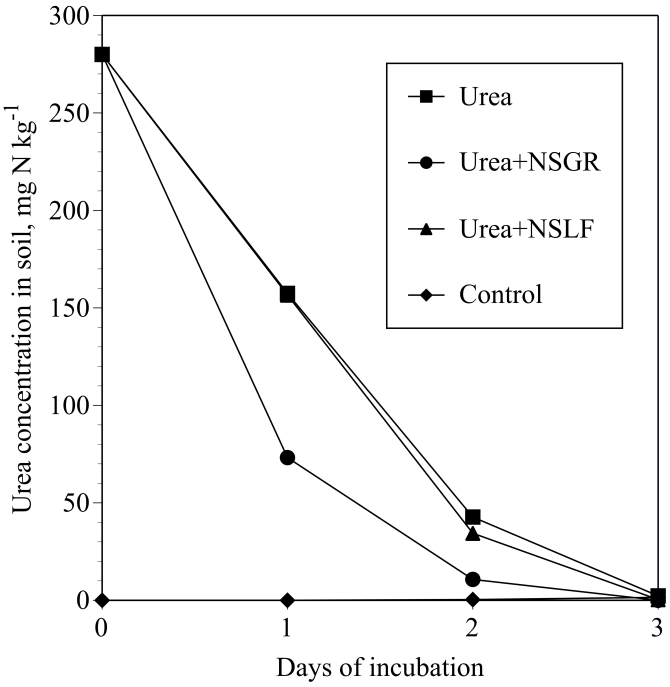

Figure 1. Urea remaining in an Overly soil, as influenced by time of incubation, and application of urea, urea plus Nutrisphere-N for granular fertilizers (NSGR), and urea plus Nutrisphere-N for liquid fertilizers (NSLF) (experiment by R.J. Goos; cited in Franzen et al., 2011).

Table 6. Cumulative ammonia volatilization losses for urea, ammonium sulfate, urea + NBPT, and urea + 0.25% Nutrisphere (NSN) from a Dewitt silt loam soil during a 15-day laboratory incubation at 25°C. (Norman data, University Arkansas, Fayetteville) (from Franzen et al., 2011).

	Days after N source application			
	3	7	11	15
N sources	Cumulative NH_3 loss, % of N applied			
Urea	14.5	35.9	51.8	56.9
Ammonium sulfate	0.1	0.2	0.5	0.6
Urea + NBPT[†]	0.006	2.7	12.9	18.3
Urea + 0.25% NSN	17.6	42.2	57.8	62.7
LSD(0.05)[‡]	12.2			
LSD(0.05)[§]	9.6			

[†]NBPT= N-(n-butyl) thiophosphoric triamide
[‡]LSD to compare means between N sources within the same sampling time.
[§]LSD to compare means between sampling time within the same N source.

Additional studies on possible urease inhibition by Nutrisphere, and some newer products including Stay-N (Loveland, Products Inc., Greeley, CO- a calcium heteropoly saccharide/calcium aminoethylpiperazine/Alkylarylpolyoxethylene glycols product) N-Stay, N-Zone (AgXplore, similar ingredients to Stay-N) and OAC a proprietary material from Simplot with an unknown composition. The methods used in these studies are available from (Goos, 2012).

In an incubation study (Fig. 2), UAN untreated had the highest level of ammonia loss, followed by UAN treated with Nutrisphere for liquid fertilizer. UAN with ammonium thiosulfate (ATS) or calcium thiosulfate (CTS) had similar, but less ammonia loss compared to UAN with Nutrisphere, but more loss than either UAN with NBPT (Agrotain) or UAN with both Agrotain and CTS (the least loss in the trial. Having previously shown that Nutrisphere is not a urease inhibitor, the likely reason for the lower ammonia loss with Nutrisphere compared to UAN is the acidic nature of the Nutrisphere for liquid fertilizer, which would tend to retain some of the ammonia from immediate loss through forming ammonium ions after urease split the urea in the UAN. As support for this conclusion, the study was performed using granular urea and Nutrisphere for granular urea, which is not as acidic as the Nutrisphere for liquid fertilizer. The results from Nutrisphere on its ability to retain ammonia after urease activity were similar to all products except NBPT (Agrotain) (Fig. 3). Three additional products, Stay-N, N-Stay and N-Zone similarly did not inhibit urease activity as exhibited by ammonia losses similar to the check. The company that markets N-Zone has not claimed to be a urease inhibitor, but claims to inhibit nitrification.

In another series of laboratory experiments conducted in 2012, (Goos, unpublished data, presented at American Society of Agronomy meetings, Oct. 2012, Cincinnatti, OH) urease activity of Nutrisphere was reevaluated. Experiment 1 compared Nutrisphere treated UAN with UAN alone. There was no effect on urea remaining after incubation compared to the untreated UAN (Fig. 4). showed that the mode of action claimed for any urease activity by Nutrisphere was flawed. There was no indication that any of 13 organic acids, including maleic and itaconic acids, had exceptional abilities to sequester Ni ions. NBPT, however, had a relatively high ability to sequester the Ni ion, and this might contribute to the activity of this compound on its selectivity for urease enzyme. Urease activity was not affected by Nutrisphere any of six experiments conducted by Goos.

Goos performed six experiments in 2012, reported at the American Society of Agonomy meetings, October 2012, and currently submitted for publication that investigated the urease activity of Nutrisphere and other products and also investigated the alleged mode of action of maleic and itaconic acid (the active ingredients in Nutrisphere) for urease activity.

The first experiment was conducted with UAN with and without Nutrisphere. The results (Fig. 4) showed no activity of the Nutrisphere-UAN compared to UAN alone.

Experiment 2 compared urea remaining after soil incubation with Nutrisphere-treated urea pretreated by a supplier and treated by the researcher, urea alone and urea with Agrotain Ultra (an NBPT formulation). The only product that increased the concentration of urea remaining in the pots at 2 day measurements to 10 day measurements was the Agrotain Ultra (Fig. 5).

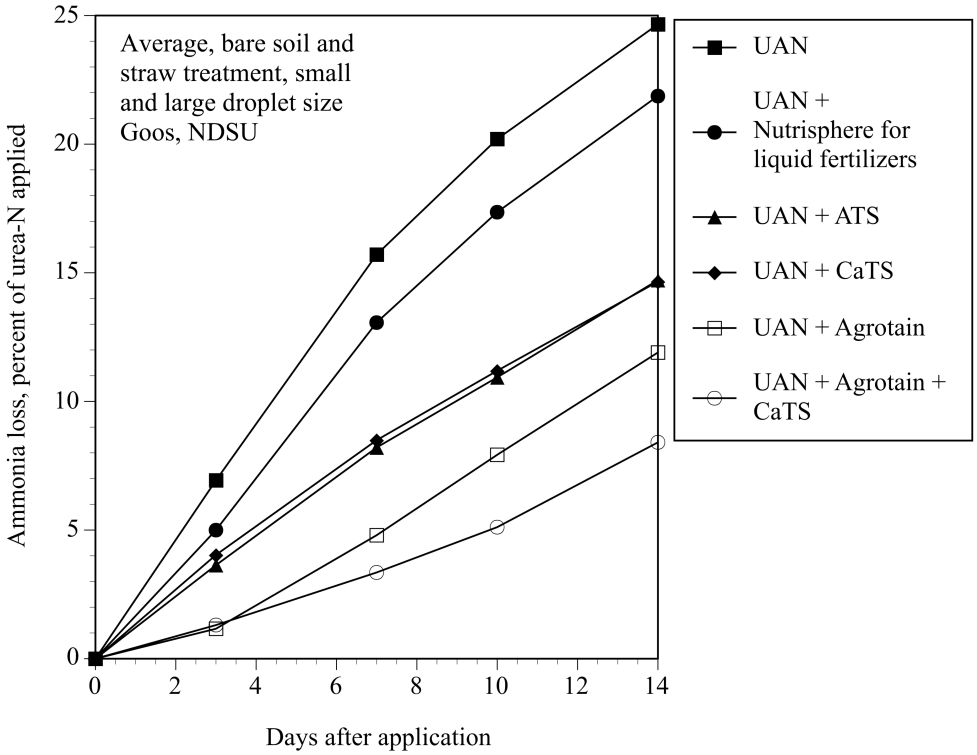

Figure 2. Ammonia loss from soil treated with UAN plus additives over 14 day incubation (from Goos, 2012).

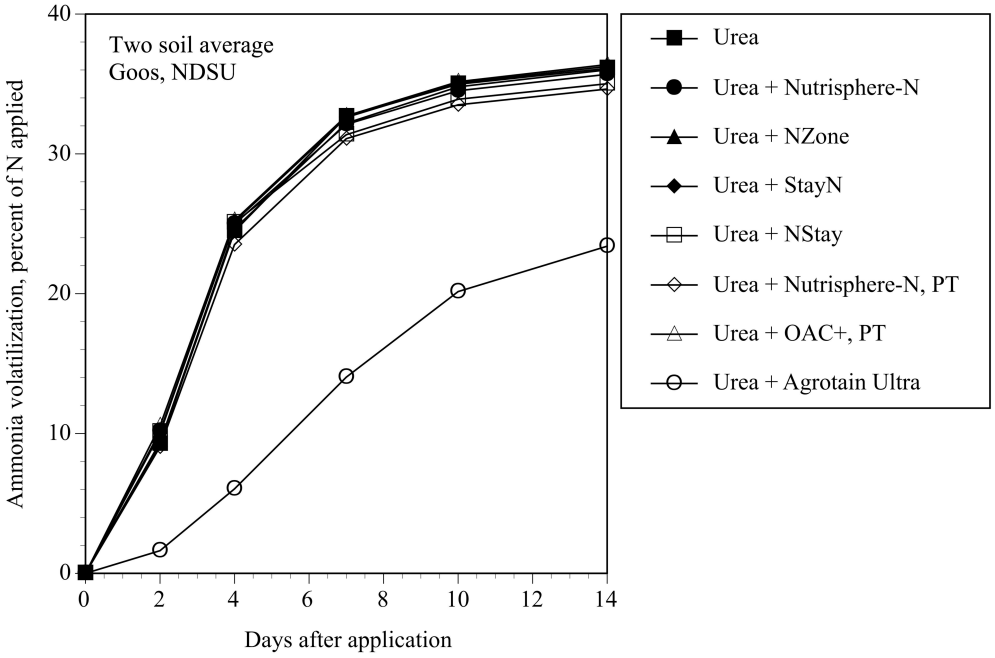

Figure 3. Ammonia loss from urea-N treated soil over 14 day incubation (from Goos, 2012).

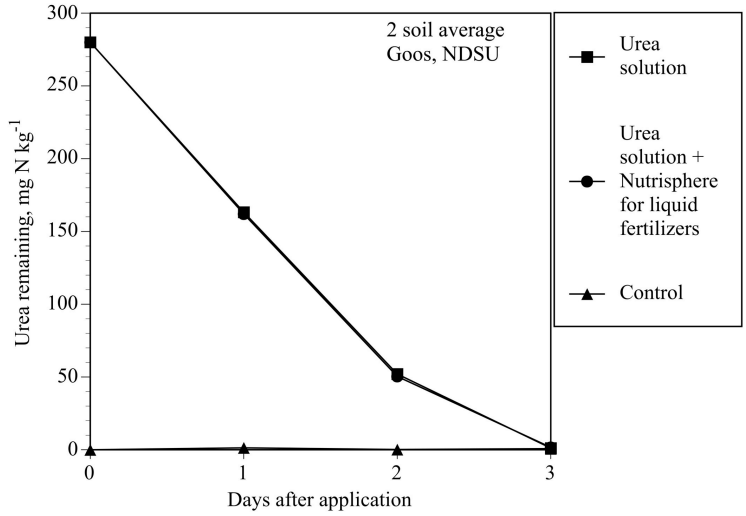

Figure 4. Urea remaining after UAN with and without Nutrisphere for liquid fertilizers, 2012 (unpublished data).

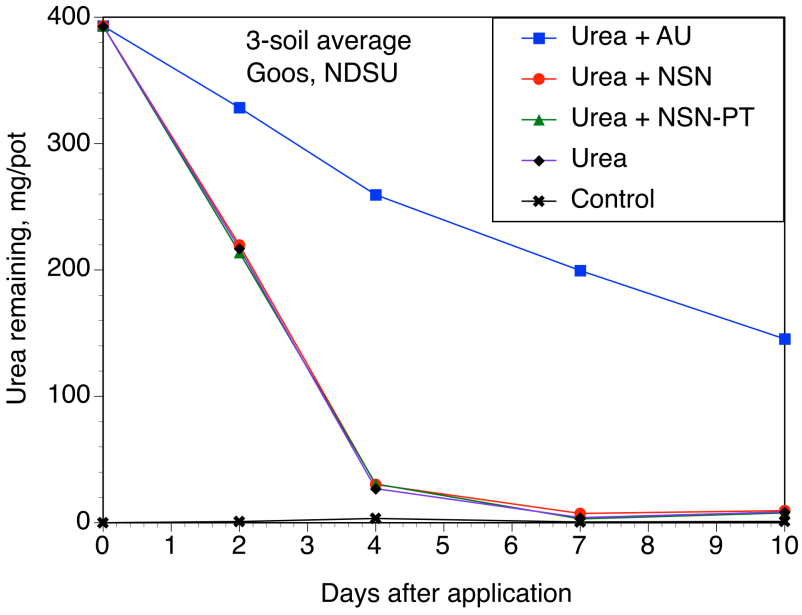

Figure 5. Urea remaining during an incubation, comparing the urease activity of urea with Agrotain Ultra (AU), urea with Nutrisphere pre-treated (PT) or treated by the researcher (NSN), or urea alone with a no-urea control.

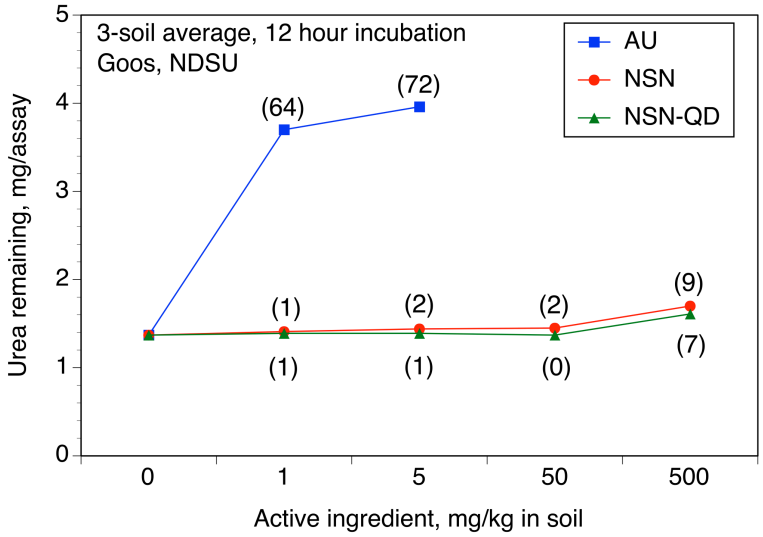

Figure 6. Urea remaining after treatment with either Nutrisphere N for urea (NSN) or Nutrisphere N Quick Dry (NSN-QD) and incubation for 12 hours at 25°C. Percent of original urea remaining is in parentheses (Goos unpublished, 2012).

In Experiment 3, 5 mg urea was mixed with 10 g soil. Urea was mixed with either then 1 to 500 mg of Nutrisphere (NSN) for urea or Nutrisphere quick dry (NSN-Q) for urea. These two Nutrisphere treatments were compared to urea alone and with 1 or 5 mg NBPT (Agrotain Ultra (AU). The soil was incubated for 12 hours at 25°C and the urea remaining was measured at the end of the experiment (Fig. 6). The Agrotain Ultra treatments of 1 or 5 mg NBPT resulted in 64% and 72% of the original urea remaining respectively. The lower NSN and NSN-QD rates of 1 and 5 mg were equivalent to labeled rates for the products. Urea remaining was less than 2% of the original rate for both treatments. Increasing the rate of NSN and NSN-QD by a factor of 10 did not improve the amount of urea remaining after incubation. Increasing the rate of NSN and NSN-QD by a factor of 100 improved the percent urea remaining to 7 and 9 respectively.

Experiment 4 was designed to test the theory of the Nutrisphere mode of action, which is to sequester Ni ions and pull Ni out of the urease enzyme, rendering it inactive. Thirteen carboxylic acids with different Ni+ stability constants were added to 5 mg urea at 50 mg/kg along with NSN, NSN-QD at 50 mg maleic/itaconic acid equivalent and AU at 1 and 5 mg/kg NBPT. The urea with and without additives were added to 10 g soil of three soils and allowed to incubate at 25°C for 12 hours. The urea remaining was then analyzed. NSN and NSN-QD were no more effective in urease inhibition compared to the thirteen carboxylic acids. AU at both rates had similar amounts of urea remaining compared with the results from Experiment 3 (Table
NSN and NSN-QD (Table 7).

Table 7. Experiment 4 treatments to test thirteen carboxylic acids, including the active ingredients in Nutrisphere was Nutrisphere for urea (NSN), Nutrisphere-Quick Dry (NSN-QD) and Agrotain Ultra (NBPT) (Goos, unpublished 2012).

Test inhibitor	Ni^{2+} stab. const. log K	Urea remaining mg	Percent inhibition %
None	--	1.07	--
Itaconic acid	1.8	1.17	2
Maleic acid	2.0	1.23	4
Malic acid	3.2	1.10	1
Oxalic acid	5.3	1.13	2
Citric acid	5.4	1.12	1
Salicylic acid	7.0	1.12	2
Imidodiacetic acid	8.1	1.14	2
NTA	11.5	1.11	1
EGTA	13.5	1.08	0
HEDTA	17.1	1.02	-1
EDTA	18.5	1.04	-1
DTPA	20.2	1.07	0
CDTA	20.2	1.04	-1
NSN	--	1.06	0
NSN-QD	--	1.04	-1
NBPT, 1 mg/kg	--	3.52	62
NBPT, 5 mg/kg	--	3.88	72
Except as noted, all materials added at 50 mg/kg			

Experiment 5 was designed to test the activity of Nutrisphere and NBPT (Agrotain Ultra) on urea with a urease solution. 5 mL of THAM (tris(hydroxymethy)aminomethane), an organic buffer (0.2M, pH7), 5 mL jackbean urease solution, and 5 ml test inhibitor (4 mg/L NBPT as AU, 40 mg/L maleic/itaconic acid as NSN or NSN-QD were combined and shaken for 1 hour at room temperature; 5 mL of urea substrate (200mg/L) was added. The urea remaining from each treatment was measured after 5, 30, 60, 90 and 120 minutes of shaking (Fig. 7). Agrotain Ultra resulted in nearly all of the urea recovered. NSN and NSN-QD appeared to recover even less urea than the control, suggesting that urease activity might have been enhanced by their activity.

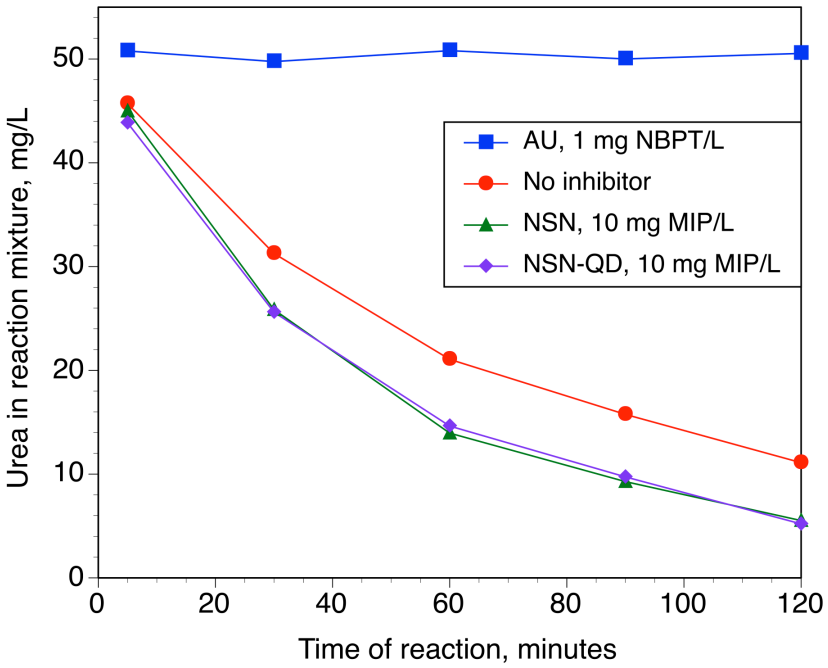

Figure 7. Amount of urea remaining in a jackbean urease solution with Agrotain Ultra (NBPT), Nutrisphere for urea (NSN) or Nutrisphere Quick Dry (NSN-QD) after reaction at 5, 30, 60, 90 and 120 minutes (Goos, unpublished data, 2012).

Experiment 6 tested the effect of NBPT (Agrotain Ultra) and maleic/itaconic acid (Nutrisphere for urea and Nutrisphere Quick Dry) concentration on urease activity. Five millilieters THAM buffer, 5 mL jackbean urease solution and 5 mL test inhibitor were mixed (0-04 to 4 mg/L NBPT as Agrotain Ultra; 0.04 to 400 mg/L maleic/itaconic acid (NSN or NSN-QD) and shaken for 1 hour; 5 mL urea substrate (200 mg/L) was added and shaken for 120 minutes. Urea remaining was analyzed.

The AU treatments exceeded the control in urea remaining at all concentrations of NBPT (Fig.8). The 1 mg/L treatment contained nearly all of the original urea. The NSN and NSN-QD treatments recovered no more urea than the control. The 10 mg/L and 100 mg/L treatments appeared to decrease the urea remaining compared to the control.

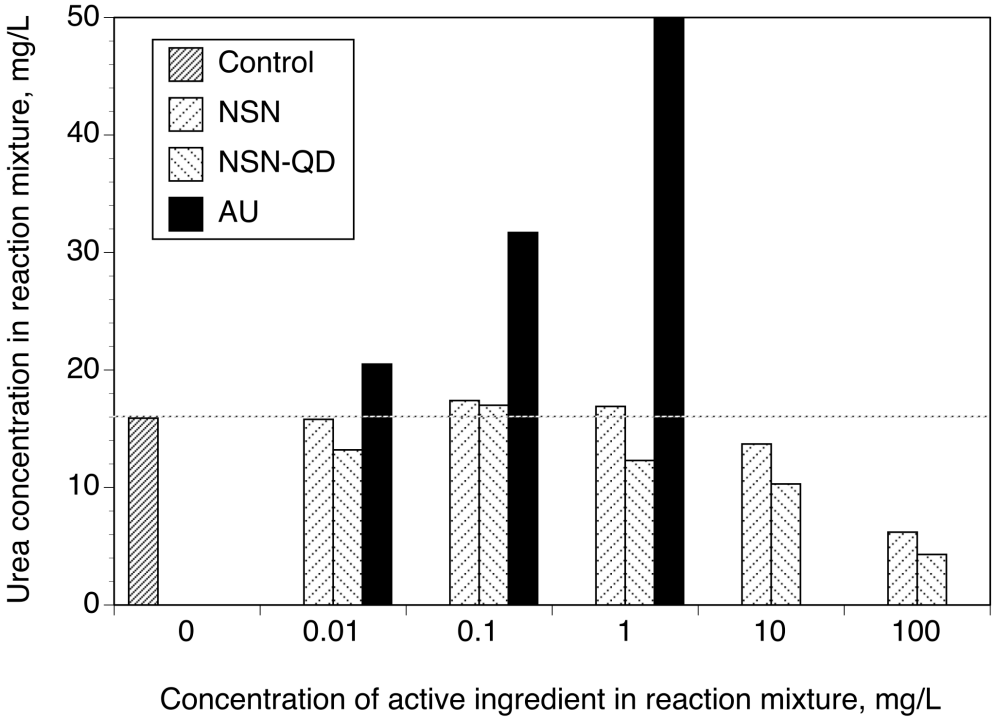

Figure 8. Urea remaining (50 mg/L original) treated with 0.01, 0.1 and 1 mg/L NBPT (Agrotain Ultra-AU), and 0.01, 0.1, 1, 10 and 100 mg/L maleic/itaconic acid as Nutrisphere for urea (NSN) and Nutrisphere Quick Dry (NSN-QD) after reaction with jackbean urease for 2 hours.

It is clear from the laboratory experiments that there is no urease inhibition by Nutrisphere when used at label rates or even greatly increased rates. In the field, it is uncommon to consistently find yield or quality responses to the use of Nutrisphere at labeled rate. In North Dakota studies on spring wheat at 8 locations, there were no yield increases or grain N uptake increases with Nutrisphere compared to urea (Franzen et al., 2011). Two additional North Dakota studies in corn with no yield advantage to Nutrisphere (NDSU Carrington Research and Extension Center, unpublished data). In Kansas (Tucker and Mengel, 2008), there were no increases due to Nutrisphere with UAN over UAN surface banded or injected in grain sorghum in 2007. In 2 years of corn in Kansas, there were no yield increases from the use of Nutrisphere-N UAN compared to surface applied UAN at three total sites (Weber and Mengel, 2009). In 2009, there was no response to Nutrisphere + UAN broadcast on grain sorghum compared to broadcast UAN alone in Kansas at three locations (Weber and Mengel, 2010). There was one sorghum yield increase with surface banded Nutrisphere + UAN compared to UAN surface banded alone and two non-responsive sites. The yield increase with surface band but not broadcast suggests that perhaps the acidity of the Nutrisphere may have delayed nitrification at this site (Schmidt, 1982).

At Waseca, MN in 2009(Randall and Vetsch, 2009) corn yield and stover N between urea and urea with Nutrisphere were similar. In Illinois, at two locations in 2008 Nutrisphere-urea was lower in yield than urea, and similar in yield at the two locations

with UAN and Nutrisphere-UAN (Ebelhar and Hart, 2009). At Dixon Springs in 2009, Nutrisphere urea, UAN, and ammonium sulfate treatments did not result in higher corn yield than the N sources with Nutrisphere-N (Ebelhar and Hart, 2010), although main effects for Nutrisphere-N on corn yield were significant. In Arkansas and Mississippi, Nutrisphere-N had no effect on rice yields in three field studies compared with urea (Franzen et al., 2011). In South Dakota, Nutrisphere-N did not result in higher corn yield in 2007 (Bly and Woodard, 2007), 2008 (Bly et al., 2008), or 2009 at 2 sites (Bly et al., 2009).

In Idaho, there were no spring wheat yield increases with Nutrisphere over 2 years (Jeffrey Stark, personal communication, 8/23/2010). In barley, however, there were yield increases in 2008 and 2009 with Nutrisphere, but no increase in grain protein over similar rates of urea. Plant N uptake with Nutrisphere was similar to urea without Nutrisphere, suggesting that the yield increase in barley came from some other response other than enhanced N nutrition (Stark, 2008. 2009).

In Kentucky, Nutrisphere-N urea performed similarly for corn grain yield as the urea check, while urea with Agrotain, SuperU or ESN poly-coated urea had higher corn yield than the check and yields were similar to those achieved with ammonium nitrate.

Laboratory studies with Nutrisphere-N show no effect on nitrification or urease activity. Therefore, it is not surprising that the great majority of studies with Nutrisphere show no yield effects. What is surprising is that there are studies that show yield effects, but not from increased N nutrition. The results from Gordon (2008) suggest that under some conditions, Nutrisphere may have some effect on plant growth and development and even N nutrition not related directly to urease inhibition or nitrification. However, the company probably needs to reexamine its label as a urease inhibitor.

Summary

Certain nitrogen additives provide growers with options for extended activity of nitrogen Availability to their crops. Their economics depends on rainfall following application, application methods, timing and soil characteristics, especially soil texture. NBPT (Agrotain) is an effective urease inhibitor. Thiosulfates have shown some urease inhibition characteristics, but again, the body of literature that supports their use is small.

Nutrisphere has been shown to be ineffective as a urease inhibitor. The data that support the use of Nutrisphere is small in comparison to the data that does not support its use. If one accepts that the laboratory studies, conducted in a similar manner to those used to evaluate products like Agrotain, show that Nutrisphere is not a nitrification or a urease inhibitor, than there must be other explanations for small number the field studies that show a yield benefit to the use of the product and in some circumstances even show an accumulation of N. Stay-N and NStay have failed to support their claims or urease inhibition in careful laboratory experiments.

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