FIELD AND LABORATORY STUDIES COMPARING NUTRISPHERE-NITROGEN UREA WITH UREA IN NORTH DAKOTA, ARKANSAS, AND MISSISSIPPI

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□ *Nitrification and ammonia volatility are two important impediments to nitrogen (N) use efficiency and crop uptake around the world. Nutrisphere® is a relatively new product whose manufacturer claims both nitrification and urea volatilization inhibiting properties. Urea coated with Nutrisphere is and the resulting fertilizer is called Nutrisphere®-N urea, or Nutrisphere-N (NSN). Eight field studies on spring (*Triticum aestivum *L.) or durum [*T. *turgidum *L. subsp* duram (Desf.) Husn.] wheat in North Dakota, three field studies in Mississippi/Arkansas on rice (*Oryza sativa *L.), four laboratory experiments in North Dakota and one in Arkansas were conducted to determine the nitrification and urea volatilization inhibiting ability of NSN compared with urea alone. Results of field and laboratory experiments revealed that the product has no nitrification or urea volatilization inhibiting properties at the recommended rates and spring wheat and rice did not benefit from the application of NSN to urea.*

Keywords: nitrogen, rice, wheat, fertilizers, soil fertility

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INTRODUCTION

Two important nitrogen (N) transformation processes in soils are volatility of ammonia from urease activity on fertilizers containing urea and nitrification. Urea volatilization has been extensively reviewed (Bock and Kissel, 1988; Kissel et al., 2008). Nitrification has likewise been extensively reviewed (Norton, 2008). Nitrapyrin has been used to delay conversion of ammonium to nitrate for over thirty years (Touchton et al., 1978; Touchton et al., 1979; Shi and Norton, 2000) and is the most commercially used product for this purpose (Bronson, 2008), although it has the disadvantage of being volatile and must be applied below the soil surface. Nitrapyrin is currently marketed as N-Serve® by Dow AgroSciences (Indianapolis, IN, USA). Over twenty compounds were tested for effectiveness as a nitrification inhibitor by Bundy and Bremner (1973), including 1,amino 1,2,4-triazole (ATC) and dicyandiamide (DCD), but none were as effective as nitrapyrin. The urease inhibitor of greatest current interest is n-butyl-thiophosphoric triamide (NBPT) (Rawluk et al., 2001; Trenkel, 1997), currently marketed by Agrotain International (St. Louis, MO, USA) as Agrotain®. The compound has had a high level of success in inhibiting urease activity (Hendrickson, 1992; Rawluk et al., 2001). Ammonium thiosulfate has also been recognized to reduce the rate of nitrification and urease, but not as effectively as nitrapyrin for nitrification or NBPT for urease inhibition (Goos, 1985).

Nutrisphere-N®, or NSN (Specialty Fertilizer Products, LLC, Leawood, KS, USA) is a 30–60% co-polymer of maleic and itaconic acid that, according to product literature, inhibits nitrification through complexing soil copper ions and inhibits urease activity by complexing nickel ions within the urease enzyme itself (Blaylock and Murphy, 2006; Sanders, 2007). There is remarkably little printed and no scientific refereed literature on the exact mechanisms of this compound on soil nitrification and urease activity. In theory, a compound that removes copper and nickel from their respective metalloenzyme might be expected at the proper rate to inhibit these processes. Nickel (Ni) is an essential element for plants due to its role as a constituent of the metalloenzyme urease (Dixon et al., 1975; Fishbein et al., 1976; Eskew et al., 1984). Copper (Cu) is a component of enzymes in nitrification bacteria that are responsible for the transformation of ammonium to nitrite and nitrate (Bock and Wagner, 2006). Complexing agents are currently being used or investigated to control and inhibit nitrification in waste water treatment plants (Vandevivere et al., 1998; Xu et al., 2000). Amounts of activated charcoal or zeolite required to inhibit nitrification in the Vandevivere et al. (1998) study were 0.5 g L^{-1}. There is no published evidence that Nutrisphere complexes Ni or Cu.

In North Dakota, urea is often applied to the soil surface in no-till spring wheat and durum production. In spring wheat and durum production, a single preplant nitrogen application is conducted. Urea is applied to some

fields in late fall before soil freeze-up, usually in late October. Urea is also applied in the spring to other fields prior to seeding in April or May, and sometimes, but not as often, applied immediately after seeding on the soil surface. Although surface application of urea is not recommended by University Extension specialists, it is still utilized by a large number of growers. Surface urea, both in the fall and in the spring is subject to N loss through urease activity and ammonia volatilization. In addition, fall or early spring application of urea is often subject to nitrification, followed by nitrate leaching or denitrification.

In the delayed-flood rice culture practiced in the southern USA, rice is dry-seeded, allowed to emerge and grow without ponding until the beginning tillering stage at which time a permanent flood is established and maintained until rice maturity. Immediately prior to flooding, 65 to 100% of the total N fertilizer requirement (i.e., normally 101 to 168 kg N ha^{-1}) is typically applied as urea on to a dry soil and the flood established as expeditiously as possible (Norman et al., 2003). The flood effectively incorporates the urea into the soil and halts ammonia volatilization (Savin et al., 2007). Most commercial rice producers require five to 14 days to establish a flood across a field. Substantial ammonia volatilization of urea can occur over the five to 14 days prior to flood establishment and significantly reduce rice N uptake and grain yield (Griggs et al., 2007; Norman et al., 2006, 2009). The objectives of these studies were to evaluate NSN in the field compared to urea at similar rates and to evaluate the nitrification and urease inhibiting properties compared to urea under controlled conditions in the laboratory.

MATERIALS AND METHODS

North Dakota Field Experiments

Seven field experiments were conducted in North Dakota in 2008 on spring wheat and one with durum wheat. Soil series, previous crop and variety used during the studies appear in Table 1. Results of soil analysis conducted immediately prior to treatment application are shown in Table 2. The experiments were designed as randomized complete blocks, with at least nine treatments and four replications. All studies included additional treatments not pertinent to the objectives of this paper, therefore the results for these additional treatments are not included. The treatments pertaining to the objectives of this paper consisted of the check with no supplemental N, 34 kg N ha^{-1} as urea, 67 kg N ha^{-1} as urea, 101 kg N ha^{-1} as urea, 34 kg N ha^{-1} as urea + NSN), 67 kg N ha^{-1} as urea + NSN, and 101 N kg ha^{-1} as urea + NSN. Urea was treated with Nutrisphere (Specialty Fertilizer Products, LLC, Leawood, KS, USA) by J.R. Simplot, Grafton, ND, at the rate they recommended for prilled urea. Wheat plots were at least 6-m long by 2.4-m wide. The center of each plot, usually 1.2-m wide, was harvested the length of

TABLE 1 Soil series, previous crop and variety in North Dakota field experiments

Site	Soil series	Series description	Previous crop
Valley City	Barnes loam	Fine-loamy, mixed, superactive, frigid Calcic Hapludolls	Barley
Hazelton	Williams loam	Fine-loamy, mixed, superactive, frigid Typic Argiustolls	Sunflower
Wishek	Bowdle loam	Fine-loamy over sandy, mixed, superactive, Pachic Haploborolls	Soybean
Dickinson	Mott fine-sandy loam	Coarse-loamy, mixed, superactive, frigid Typic Haplustolls	Oat
Langdon	Hamerly loam	Fine-loamy, frigid Aeric Calciaquolls	Durum
Cavalier	Bearden silty clay loam	Fine-silty, mixed, superactive, frigid Aeric Calciaquolls	Sugar beet
Williston 1	Williams loam	Fine-loamy, mixed, superactive, frigid Typic Argiustolls	Spring wheat
Williston 2	Williams loam	Fine-loamy, mixed, superactive, frigid Typic Argiustolls	Durum

the plot with a plot combine when wheat was mature and dry. Grain moisture and test weight was determined using a Dicky-John GAC 500XT moisture meter (Dickey-John, Minneapolis, MN, USA). Grain protein was determined using the Infra Tec 1126 grain protein analyzer (Dresden, Germany). Grain moisture for grain protein reporting was adjusted to 14 per cent. Statistical analysis was conducted using the GLM model within SAS 9.1 for Windows®. (SAS Institute, Cary, NC, USA).

Soil samples were obtained from the Hazelton and Langdon plot areas three weeks after treatment application. At Hazelton, three 75-mm diameter soil cores to a 15-cm depth were obtained from each plot of the four replications of the 101 kg ha^{-1} N as urea, and 101 kg ha^{-1} N as NSN. The sub-sample cores from each plot were mixed and analyzed for extractable ammonium-N and nitrate-N. At Langdon, soil sampled treatments included UAN (urea-ammonium nitrate solution, 28-0-0). The UAN treatments were

TABLE 2 Soil analysis immediately prior to N treatment, North Dakota field experiments

	Site							
Property	V*	H	W	D	L	C	W1	W2
Soil nitrate-N, ppm[†]	78	135	41	19	17	49	52	52
P[‡], ppm	13	11	3	18	16	18	9	8
OM, g kg^{-1}	36	37	24	19	51	55	22	22

*V = Valley City; H = Hazelton; W = Wishek; D = Dickinson; L = Langdon; C = Cavalier; W1 = Williston site 1; W2 = Williston site 2.

[†]Soil nitrate-N from 15-cm depth soil sample. Procedure from Gelderman and Beegle, 1998.

[‡]P and OM from 15-cm depth soil sample. Olsen P procedure from Frank et al., 1998; OM loss on ignition (Combs and Nathan, 1998).

broadcast sprayed using 8002 spray tips on the soil at the same time as urea application. The urea + N-Serve treatment was incorporated within one hour of application using a field cultivator set at a 10-cm depth. Multiple bucket-cores (75-mm diameter soil cores taken to a 15-cm depth) were obtained from the check (0 kg N ha^{-1}) and the 101 kg N ha^{-1} rate of the following treatments: i) urea, ii) urea + NSN, iii) urea + N-Serve, iv) UAN, v) UAN + + NSN, and vi) UAN + Agrotain®. The composite samples from each of the six replications were analyzed for ammonium-N and nitrate-N.

North Dakota Laboratory Experiment 1

An incubation study was performed at room temperature (20–25°C). The two soils used were an Overly clay loam (Pachic Hapludolls) and a Renshaw sandy loam (Calcic Hapludolls). The Overly soil was a clay loam derived from glacial lacustrine deposits. The Renshaw soil was a sandy loam derived from glacial outwash. The soils had pH values of 6.3–6.4. Characteristics of the two soils are shown in Table 3. Twenty five gram portions of air-dried and sieved (<2 mm) soil were placed in 100 mL plastic cups. The soils were moistened with water (6 mL for the Overly soil, 3 mL for the Renshaw soil), capped with lids containing four 1 mm holes for aeration, and placed in an incubator with elevated humidity. The soils were incubated, without fertilizer treatment, for four days, to allow the soil microflora to reestablish growth. The cups were weighed before and after the pre-incubation period, and the water lost by evaporation was negligible. The experimental design consisted of four treatments in a completely random design. The first treatment was a control, with nothing else added to the soil. The second treatment was a single 0.1 mL droplet of a urea solution applied as a spot treatment on the surface. The urea solution consisted of 150 g L^{-1} of reagent-grade urea

TABLE 3 Soil characteristics for North Dakota laboratory experiments

Measurement[†]	Unit	Overly	Renshaw
Sand	g kg^{-1}	284	769
Silt	g kg^{-1}	425	142
Clay	g kg^{-1}	291	89
Texture		cl loam	sa loam
Organic matter	g kg^{-1}	39	21
CaCO$_3$	g kg^{-1}	> 1	2.3
pH	—	6.3	6.4
EC	dS m^{-1}	0.3	0.1
CEC	cmol (+) kg^{-1}	32	10
P	mg kg^{-1}	44	8
K	mg kg^{-1}	216	90

[†]Texture by hydrometer method, organic matter by weight loss by ignition, CaCO$_3$ by pressure calcimetry, pH and conductivity of a 1:1 soil:water suspension, CEC by the sodium oxalate method, Olsen P (Frank et al., 1998), and available K (Warncke and Brown, 1998).

in water. This application method was chosen to simulate the reaction of a single, intact 15 mg urea granule (7 mg N) with moist soil under aerobic conditions. The third treatment was a 0.1 mL droplet of a solution containing 150 g L^{-1} of urea and 0.313 mL L^{-1} of a liquid formulation of Nutrisphere for application to granular urea (NSGR). The rate of NSGR was chosen to equal the ratio of Nutrisphere to urea currently recommended on the label (0.5 gallon of product per 2000 lbs of granular urea, 2.08 mL of product per kg of urea, or 0.313 mL of product per 150 grams of urea). The fourth treatment was a 0.1 mL droplet of a solution containing 150 g L^{-1} of urea and 50 mL L^{-1} of a Nutrisphere formulation for liquid fertilizers (NSLF). The rate of NSLF was chosen to coincide with the recommended rate of this formulation for liquid fertilizers, 0.5% (vol/vol). After application of the appropriate treatment, the caps were placed back on the cups, and the cups were placed in the incubator. Twenty four cups of each treatment were prepared for each soil.

After 1, 2, 3, and 4 days of incubation, three cups of each treatment were removed from the incubator, extracted for five minutes by shaking with 125 mL of 2 M potassium chloride (KCl). This procedure was imposed on one replicate at a time, so that shaking times could be kept to exactly five minutes, and equal for all treatments. A five-minute KCl extraction was suggested by Keeney and Bremner (1967) for quantitative extraction of urea from soil with minimal urea hydrolysis during extraction. The extracts were filtered, and the residual urea determined by the diacetyl monoxime method of Mulvaney and Bremner (1979).

After 7, 14, 21, and 28 days, three cups of each treatment were removed from the incubator, extracted with 2 M KCl for 1 hour, and the filtrates analyzed for ammonium by a gas diffusion/conductimetric method and for nitrate by a cadmium reduction method (Keeney and Nelson, 1982; Mansell et al., 2000). All cups remaining after the 14-day sampling were weighed and water lost by evaporation (typically 0.3 mL) was replenished by application of water to the edge of the soil surface, avoiding the spot where the fertilizer was applied.

North Dakota Laboratory Experiment 2

The second experiment measured ammonia loss from the soil surface, as influenced by granular urea and granular urea coated with Nutrisphere. The experiment was performed in a greenhouse using 15 cm diameter pots containing 2 kg of Renshaw soil. The method of trapping ammonia was similar to that used by Nommik (1973). The experimental design was a factorial of three fertilizer treatments by two straw treatments by four replicates in a completely random design. The three fertilizer treatments were an untreated control, urea granules placed on the soil surface, and urea granules coated with Nutrisphere and placed on the soil surface. Commercial urea

granules and commercial urea granules treated with the recommended rate of Nutrisphere (2.08 mL per kg of urea, or 0.5 gallon per 2000 lbs.) were provided by the J.R. Simplot Company, Grafton, ND. To assure uniform size, the granules were sieved, and granules retained on a 3.3 mm sieve were used. The rate of urea or urea + Nutrisphere granules (NSGR) was 0.405 +/− 0.005 grams per pot, which provided an addition of 91 mg N kg^{-1} of soil or about 100 kg N ha^{-1} on a surface area basis. On the average, there were 14 granules pot^{-1} for either material. The two straw treatments were a bare soil surface versus a soil surface partially-covered with wheat straw. In the bare soil treatment, 1 kg of soil was mixed with 125 mL of water in a plastic bag, allowed to equilibrate for a few hours, mixed again, and placed in a pot, with a small (2.5 cm diameter) rigid plastic tube placed vertically in the center of the pot for later application of water to the bottom of the pot. A second layer of 1 kg of soil, moistened as described above, was then layered on top of the first layer. The soil + straw treatment was prepared by mixing 1 kg of dry soil and 125 mL of water in a plastic bag, and placing in the bottom of the plastic pot, again with a tube for later application of water to the bottom of the pot. A second layer of moistened soil, 850 g of dry soil plus 105 mL of water, was placed in the pot on top of the first layer of soil. A third layer of soil was prepared by mixing 9.1 g of ground (<0.5 mm) wheat straw with 150 g of soil and 20 mL of water in a plastic bag, and layering this mixture on top of the moistened soil already placed in the pot. This resulted in about half of the soil surface covered with straw. For both straw treatments, a layer of soil was settled by gentle tapping of the bottom of the pot on the greenhouse bench before the next layer was added. All pots were then weighed and the weight recorded on each pot. The pots were covered with sponges moistened with water, as described below, and allowed to incubate for four days before the fertilizer treatments were applied. After the four-day pre-incubation period, the sponges were removed, the pots adjusted to the original weight by application of water down the tube to the bottom of the pot, the fertilizer pellets applied randomly on the soil surface, and the sponges replaced with sponges moistened with a phosphoric acid-glycerol solution. The sponges were made of 2.5 cm thick poly foam, about 16 cm diameter. The sponges were prepared for use by soaking in 0.1 M hydrochloric acid (HCl), followed by repeated rinsing with distilled water, wringing to remove excess water, followed by air drying. For the 4-day pre-incubation period, the sponges were soaked in water and the excess removed by repeated and forceful wringing. For the purpose of trapping ammonia, the sponges were soaked in a phosphoric acid-glycerol solution (100 mL concentrated phosphoric acid and 125 mL glycerol, diluted to 2.5 L with water). After forceful, repeated wringing to remove excess liquid, each sponge retained an average of 20 mL of liquid. The sponges were placed inside the rim of each pot, about 5 cm above the soil surface. Two, 4, 6, 9, 12, and 16 days after fertilizer application, the acid-sponges were removed, the

pots adjusted to the original weight by application of water into the tubes reaching the bottom of the pot (typically 20–30 mL), and fresh acid-treated sponges were installed. After 16 days, no sponges were placed over the pots, and the pots were allowed to dry for 1 week. The soil was mixed well, spread thinly to complete air-drying. The soil was crushed to pass a 2 mm sieve, mixed, 100 g extracted with 500 mL of 2 M KCl, and filtered. The filtrates were analyzed for ammonium and nitrate as described above. The phosphoric acid-treated sponges were extracted with 250 mL of 0.1 M HCl and the ammonium content determined by steam distillation with sodium hydroxide (NaOH) (Bremner and Mulvaney, 1982).

North Dakota Laboratory Experiment 3

This experiment compared the nitrification rate of intact commercial urea granules with and without coating with Nutrisphere. The experiment was conducted in a greenhouse using 15 cm diameter plastic pots containing 2 kg of Renshaw soil. Air-dried soil was weighed in 500 g lots into plastic bags and moistened with 60 mL of water. The bags were closed, the contents mixed well, allowed to equilibrate overnight, and mixed again. The experimental design was three fertilizer treatments by four replicates by four lengths of incubation, in a completely random design. The three fertilizer treatments were a control, commercial urea granules, and commercial urea granules coated with Nutrisphere, as described in Experiment 2. The urea and urea + Nutrisphere (NSGR) were applied at a rate of 0.405 +/− 0.005 g pot^{-1}. On the average, there were 14 granules pot^{-1} for both materials. One bag of moistened soil was emptied into the bottom of the pot and 4–5 fertilizer granules placed on the soil surface. A second layer of moistened soil was placed over the granules and 4–5 granules placed on the soil surface. A third layer of moistened soil was placed over the fertilizer granules and the remaining fertilizer granules placed on the soil surface. A fourth layer of moistened soil was placed over the fertilizer granules. After adding each layer of soil, the soil was settled in the pot by gentle tapping of the bottom of the pot against the greenhouse bench. The pots were weighed, and the weight recorded on the side of the pot. A foam sponge, moistened with water as in Experiment 2, was placed into the top of the pot, and the pots incubated. The sponges were removed twice weekly, the pots weighed, and brought to the original weight by application of water uniformly across the soil surface, typically 20–30 mL. The sponges were saturated with water, the excess removed by forceful wringing, and placed back into the top of the pot. After 7, 14, 21, and 28 days, four pots of each treatment were taken, and the soil was spread thinly, air dried, crushed to pass a 2 mm sieve, and mixed. One hundred grams of soil from each pot were extracted with 500 mL of 2 M KCl and the ammonium and nitrate content

of the extracts determined as described for Experiment 1. Statistical analysis was conducted using SuperAnova (1991, Abacus Concepts, Piscotaway, NJ, USA).

Mississippi and Arkansas Field Trials

Field studies with delayed-flood rice were conducted in 2006 at the Mississippi State University Delta Research and Extension Center (DREC) in Stoneville, MS on a Sharkey clay (Vertic Haplaquepts) and in 2007 at two University of Arkansas experiment stations, the Rice Research and Extension Center (RREC) near Stuttgart, AR on a Dewitt silt loam (Typic Albaqualfs) and the Lake Hogue Research Farm (LHRF) near Weiner, AR, on a Hillemann silt loam (Glossic Natraqualfs). Soybean was the previous crop at all three rice study locations. Soil nutrients other than N at the Mississippi site were sufficient for optimum rice growth according to Mississippi State University guidelines (Buehring et al., 2008; Table 4). Soil-test data from the LHRF and RREC study sites indicated a need for phosphorus (P) and potassium (K). Consequently, 20 kg P ha^{-1} as triple superphosphate and 50 kg K ha^{-1} as muriate of potash were applied before seeding according to University of Arkansas guidelines (Slaton, 2001). The soil at the LHRF site had a pH > 6.0 in combination with a relatively low zinc (Zn) concentration. Thus, granular-Zn fertilizer (11 kg Zn ha^{-1}) was applied as zinc sulfate (ZnSO$_4$) prior to seeding to prevent Zn deficiency. The cultivar chosen for the 2006 Mississippi study at the DREC was Cheniere and the cultivar chosen for the 2007 Arkansas studies at the LHRF and RREC was Wells. Both cultivars are long grains with excellent yield potential and two of the principal cultivars grown in the Southern USA Ricebelt. Rice was seeded at 112 kg ha^{-1} in nine-row plots (18 cm spacing) of 4.6 m in length. The rice was grown upland until the beginning tillering growth stage at which time a permanent flood (5–10 cm depth) was established and maintained until maturity. The Mississippi study was a factorial arrangement of treatments that consisted of two N rates (101 and 168 kg N ha^{-1}) plus a control (0 kg N ha^{-1}), two application times (1 and 10 days before flooding, DBF), and three N sources [urea, urea

TABLE 4 Selected soil chemical properties for the Arkansas and Mississippi experiments

Site[†]	Soil pH	Mehlich-3 extractable nutrients (mg kg^{-1})						
		P	K	Ca	Mg	S	Cu	Zn
DREC	7.3	62	424	4430	1057	15	1.9	6.5
LHRF	6.3	18	85	1098	178	8	1.5	3.7
RREC	5.9	23	125	960	136	6	3.0	6.4

[†]Site abbreviations: DREC = Delta Research and Extension Center near Stoneville, MS; LHRF = Lake Hogue Research Farm near Waldenburg, AR; and RREC = Rice Research and Extension Center near Stuttgart, AR.

treated with Nutrisphere on a 0.25% w/w basis (urea + 0.25% NSN), and urea treated with Nutrisphere on a 0.50% w/w basis (urea + 0.50% NSN)] arranged in a randomized complete block design and replicated four times. The Arkansas studies were a factorial arrangement of treatments that consisted of two N rates (67 and 134 kg N ha^{-1}) plus a control (0 kg N ha^{-1}), three application times (1, 5 or 6 and 10 or 11 DBF) and two N sources (urea and urea + 0.25% NSN) arranged in a randomized complete block design and replicated four times. All urea sources treated with Nutrisphere were prepared by Specialty Fertilizer Products, LLC (Leawood, KS, USA) at the rates they specified and recommended. Dates when selected management practices were conducted for all three field studies are shown in Table 5. At maturity, a 2.6-m^2 area from the center four rows of each plot was harvested for grain yield using a small plot combine. The harvested grain was weighed, analyzed for moisture content and the reported grain yields adjusted to a uniform moisture content of 120 g H$_2$O kg^{-1} before statistical analysis. Analysis of variance was performed on the grain yield data utilizing SAS 9.1 (SAS Institute, Cary, NC, USA) and differences among means were compared using Fisher's protected least significance difference (LSD) procedure at a $P < 0.05$ probability level.

Arkansas Laboratory Experiment

An ammonia volatilization study was conducted in the laboratory at 25°C utilizing diffusion chambers and boric acid traps as described by Khan et al. (2001). One hundred grams of the Dewitt silt loam soil (20% volumetric water content), taken from the no N control plots of the field study at the RREC, was placed in the chambers and the granular N sources were placed on the soil surface at an N-fertilizer rate equivalent of 202 kg N ha^{-1}. The four granular N fertilizer sources studied were: i) urea, ii) ammonium sulfate, iii) urea + NBPT, and iv) urea + 0.25% NSN. Agrotain solution contains the urease inhibitor NBPT at a concentration of 200 g kg^{-1} (Agrotain International, St. Louis, MO). The urea + NBPT source was prepared by blending 1 kg of prilled-urea with 5.0 mL of Agrotain solution. The urea + 0.25% NSN source was treated with Nutrisphere by Specialty Fertilizer Products, LLC at the rate they recommended for prilled urea. Ammonia volatilization of the N sources was measured at 3, 7, 11, and 15 days after N fertilizer application by replacing the boric acid traps. At each sampling time the boric acid in the traps was removed and the ammonium-N in the trap quantified by acidimetric titration according to Mulvaney (1996). The experimental design was a randomized complete block of the four N sources with three blocks and a split-plot treatment structure in which the whole plot was N source and the sub-plot was sampling time. Ammonia volatilization means were separated

TABLE 5 Pertinent agronomic information for the field studies conducted in 2006 at the Delta Research and Extension Center (DREC) and 2007 at the Lake Hogue Research Farm (LHRF) and Rice Research and Extension Center (RREC)

Practice	DREC	LHRF	RREC
Planting dates	5/9	5/10	4/19
Emergence dates	5/19	5/19	4/28
Preflood N dates,	6/1 = 10 d before flood applied	6/1 = 11 d before flood applied	5/14 = 10 d before flood applied
Precipitation dates	6/9 = 1 d before flood applied	6/7 = 5 d before flood applied	5/15 = 5.1 cm precipitation
and amounts		6/10 = trace of precipitation	5/18 = 6 d before flood applied
		6/11 = 1 d before flood applied	5/24 = 1 d before flood applied
Flood dates	6/10	6/12	5/25
Harvest dates	9/9	9/14	8/29

using LSD at $P < 0.05$ following a significant F-test in the analysis of variance. All analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

North Dakota Field Trial Results

Wheat yield increased from N fertilization at Valley City, Langdon and Cavalier (Table 6). Yield tended to decrease with the highest N rates at Williston 1. Within each location, there were no differences in yield between similar rates of urea and urea treated with Nutrisphere (NSN). Grain protein increased numerically and sometimes significantly with N rate at all locations with the exception of Hazelton (Table 7), suggesting that NSN did not function as a nitrification inhibitor. There were no differences between similar N rates of urea and NSN in grain protein except at the 101 kg N ha^{-1} rate at Cavalier, where NSN was significantly higher than untreated urea. An analysis of total grain N content at this site revealed no differences in total grain N content between these two treatments.

The soil core study of residual nitrate and ammonium-N at Hazelton showed no differences between urea and NSN applied at 101 kg N ha^{-1}. Three weeks after N application soil inorganic N forms averaged 28.3 and 27.5 mg nitrate (NO$_3$)-N kg^{-1} and 42.0 and 40.8 mg ammonium (NH$_4$)-N kg^{-1} for urea and NSN respectively. The soil core study at Langdon showed no difference between the nitrate- and ammonium-N concentrations in the untreated urea and NSN treatments. However, ammonium-N increased with N-Serve$^{®}$ treated urea (Table 8) compared with urea alone. Again, there was no indication that NSN was active as a nitrification inhibitor.

TABLE 6 Wheat yield from North Dakota field sites with N treatment

Site	Check	34-U*	34-NSN	67-U	67-NSN	101-U	101-NSN	LSD (0.05)
					Yield, Mg ha^{-1}			
Valley City	3.80	4.39	3.89	4.53	4.48	4.54	4.72	0.64
Hazelton	2.45	2.59	2.35	2.63	2.49	2.79	2.51	NS†
Wishek	1.63	1.94	1.88	1.69	1.73	1.97	1.92	NS
Dickinson	0.61	0.69	0.70	0.69	0.71	0.70	0.61	NS
Langdon	2.60	3.96	4.27	4.65	4.87	5.56	4.74	0.64
Cavalier	5.05	5.56	5.47	5.81	5.53	5.76	5.61	0.42
Williston 1	0.93	0.93	0.97	0.92	0.88	0.79	0.80	0.13
Williston 2	0.42	0.43	0.40	0.36	0.49	0.44	0.46	NS

*34-U = 34 kg ha^{-1} N as urea; 34-NSN = 34 kg ha^{-1} N as NSN; 67-U = 67 kg ha^{-1} N as urea; 67-NSN = 67 kg ha^{-1} N as NSN; 101-U = 101 kg ha^{-1} N as urea; 101-NSN = 101 kg ha^{-1} N as NSN.

†NS = no significance.

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TABLE 7 Wheat protein from North Dakota field sites with N treatment.

Site	Check	34-U	34-NSN	67-U	67-NSN	101-U	101-NSN	LSD (0.05)
				Treatment				
				Protein, g kg^{-1}				
Valley City	139	144	136	146	144	146	149	9
Hazelton	182	185	179	185	185	187	187	NS
Wishek	129	143	144	146	153	156	157	15
Dickinson	134	159	158	166	169	171	176	10
Langdon	102	102	105	106	105	114	110	5
Cavalier	117	128	128	137	132	139	145	6
Williston 1	96	165	157	184	185	194	192	9
Williston 2	190	211	211	220	217	218	223	7

*34-U = 34 kg ha^{-1} N as urea; 34-NSN = 34 kg ha^{-1} N as NSN; 67-U = 67 kg ha^{-1} N as urea; 67-NSN = 67 kg ha^{-1} N as NSN; 101-U = 101 kg ha^{-1} N as urea; 101-NSN = 101 kg ha^{-1} N as NSN.

[†]NS = no significance.

North Dakota Laboratory Experiment 1 Results

Urea remaining in the Overly soil, as a function of time of incubation, is shown in Figure 1. Urea hydrolysis was vigorous, and no measurable urea remained in the soil for any of the treatments after 3 days of incubation. There was no indication that amending the urea solution with NSGR or NSLF inhibited urea hydrolysis (Table 9, Figure 1). Interestingly, urea hydrolysis may have actually been stimulated by the addition of NSGR after 1 or 2 days of incubation. Ammonium- and nitrate-N levels in the Overly soil are shown in Figure 2. Nitrification was rapid, and after two weeks of incubation, very little ammonium-N remained in the soils treated with urea, urea + NSGR, or urea + NSLF. There was no indication that either formulation of Nutrisphere-N inhibited nitrification (Table 9), despite the application of the fertilizers as

TABLE 8 Nitrate and ammonium in soil at Langdon 3 weeks following fertilizer application

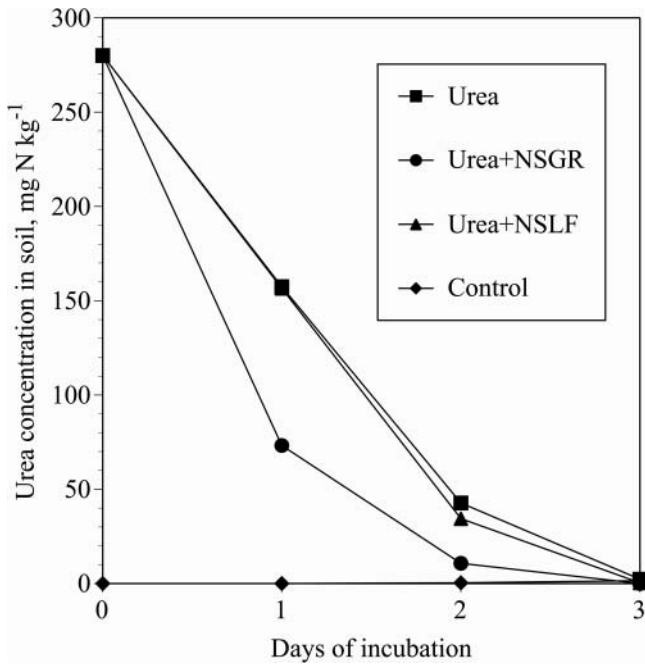| Treatment	ppm	
	Nitrate	Ammonium
Check	10.2	3.6
101 kg ha^{-1} N-urea	45.3	10.6
101 kg ha^{-1} N-NSN	40.3	8.4
101 kg ha^{-1} N Urea + N-Serve®	37.0	18.0
101 kg ha^{-1} N-UAN*	27.1	11.7
101 kg ha^{-1} N-UAN-NSN	27.3	11.9
101 kg ha^{-1} N UAN + Agrotain®	24.8	11.6
LSD (0.05)	8.4	4.5

*urea-ammonium nitrate 28-0-0 solution.

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). North Dakota laboratory experiment 1.

FIGURE 2 Ammonium and nitrate extracted from 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). North Dakota laboratory experiment 1.
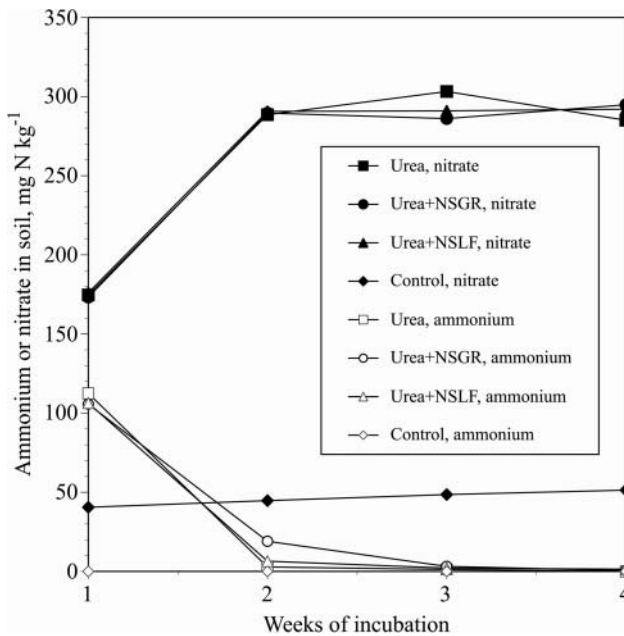
TABLE 9 Analysis of variance for soil nitrogen measurements in North Dakota laboratory experiments 1 and 3

| Measurement | Time of incubation | Significance of F | | | |
		Soil	Treatment	Soil × Trt	SE[†]
		Experiment 1			
Urea	1 d	**	**	**	4
	2 d	**	**	**	6
	3 d	+	*	**	1
Ammonium	1 wk	**	**	**	3
	2 wk	**	**	**	4
	3 wk	**	**	**	6
	4 wk	**	**	**	2
Nitrate	1 wk	**	**	**	3
	2 wk	**	**	**	10
	3 wk	**	**	**	6
	4 wk	**	**	**	2
		Experiment 3			
Ammonium	1 wk	—	**	—	1
	2 wk	—	**	—	1
	3 wk	—	**	—	1
	4 wk	—	**	—	1
Nitrate	1 wk	—	**	—	2
	2 wk	—	**	—	4
	3 wk	—	**	—	2
	4 wk	—	**	—	1

[†]SE = standard error.

+, *, ** Significant at the 0.1, 0.05, and 0.01 level, respectively.

a concentrated spot application with very minimal water movement after application.

Urea remaining in the Renshaw soil, as a function of time of incubation, is shown in Figure 3. Urea hydrolysis was somewhat slower for this soil than for the Overly soil, but a negligible (<15 mg urea-N kg^{-1}) amount of urea was recovered after three days of incubation. There was no indication that amending the urea solution with NSGR or NSLF inhibited urea hydrolysis (Table 9). Ammonium and nitrate levels in the Renshaw soil are shown in Figure 4. Nitrification was slower for this soil than for the Overly soil, but nitrification was still almost complete after four weeks. The curves for the accumulation of nitrate, or the depletion of ammonium, were similar for the urea, urea + NSGR, and urea + NSLF treatments. There was no indication that amending urea with either formulation of Nutrisphere-N inhibited nitrification.
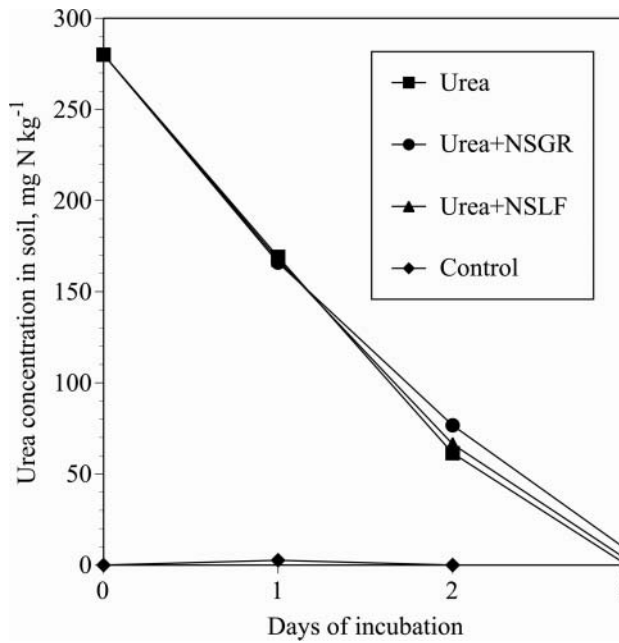
FIGURE 3 Urea remaining in a Renshaw soil, as influenced by time of incubation, and application of urea, urea plus Nutrisphere-N for granular fertilizers (NSGR), and urea plus Nutrisphere-N for liquid fertilizers (NSLF). North Dakota laboratory experiment 1.

FIGURE 4 Ammonium and nitrate extracted from a Renshaw 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). North Dakota laboratory experiment 1.
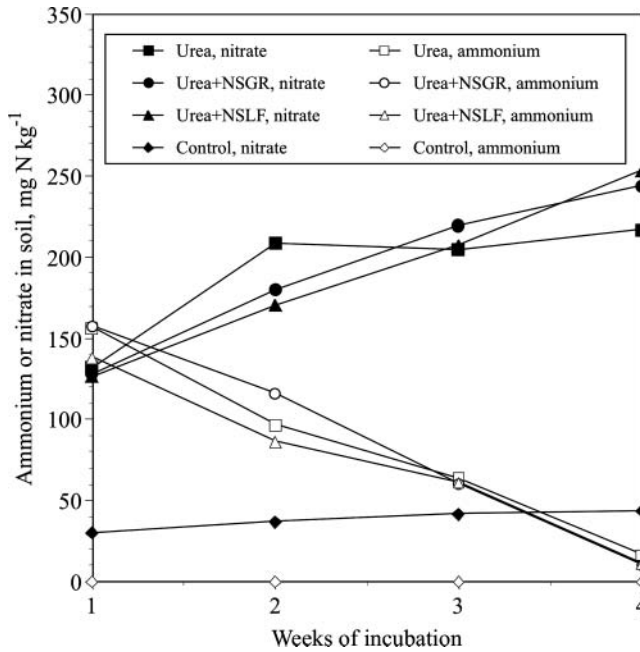
North Dakota Laboratory Experiment 2 Results

Ammonia volatilization from the surface of a Renshaw soil, as influenced by straw, urea, and urea + NSGR, is shown in Table 10. In the absence of added straw, ammonia volatilization was the greatest for the 2–4, 4–6, and 6–9 day periods. With the 0–2 and 2–4 day samplings, ammonia loss was greater from the pots treated with straw than from the pots without straw, but from 4–6 and 6–9 days the reverse was true. These trends can be more easily visualized in Figure 5. Ammonia volatilization, expressed as a percentage of the N applied, was severe, exceeding 40% in all pots receiving fertilizer. Ammonia loss was slightly less from the urea + NSGR than from the untreated urea. The reduction in ammonia loss was about 1% of the added N without straw and 2% of the added N with straw. These data are not necessarily in conflict with those reported by Sanders et al. (2003, 2004). In their studies, urea granules were coated with 10–50 grams of dry polymer equivalent per kg of urea and they observed a reduction of ammonia loss of about 50%. In Experiment 2, urea was treated with about 1 gram of dry polymer equivalent per kg of urea and the reduction of ammonia loss was 1–2% of the added N. Perhaps the rate of polymer needed to reduce ammonia volatilization is inadequate at the current Specialty Fertilizer Products recommended rate of application.

Residual ammonium- and nitrate-N in the soil after the ammonia loss period is shown in Table 10. Nitrification was not complete after 16 days, as
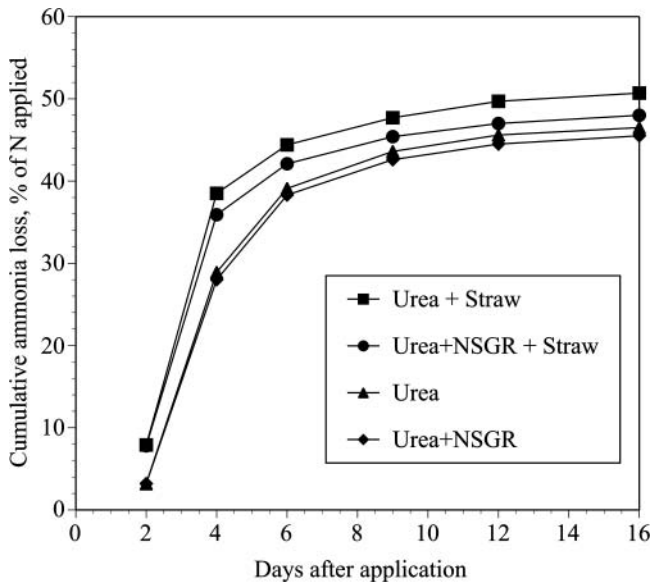

FIGURE 5 Ammonia loss from a Renshaw soil, as influenced by straw, urea, and urea treated with Nutrisphere-N for granular fertilizers (NSGR). North Dakota laboratory experiment 2.

TABLE 10 Effect of straw, urea, and urea treated with Nutrisphere-N (NSGR) for granular fertilizers on ammonia volatilization from the soil surface, and mineral nitrogen remaining in the soil. North Dakota laboratory experiment 2

Treatment	Added straw	Ammonia volatilization from the soil surface (mg N pot^{-1})						Mineral N remaining in soil (mg N kg^{-1})		
		0–2 d	2–4 d	4–6 d	6–9 d	9–12 d	12–16 d	0–16 d	Ammon.	Nitrate	Sum
Control	No	0.7	1.2	1.2	0.9	1.1	1.2	6.3	2	46	48
Urea	No	6.7	48.2	19.9	9.1	4.9	2.8	91.6	12	76	88
Urea+NSGR	No	6.6	46.7	20.0	8.8	4.6	3.0	89.7	10	74	84
Control	Yes	0.6	0.9	0.9	0.7	1.1	1.0	5.2	2	39	41
Urea	Yes	15.2	56.9	11.7	6.6	4.7	2.9	98.0	7	65	72
Urea+NSGR	Yes	15.0	52.3	12.3	6.6	4.0	3.0	93.2	6	63	69
Significance of F											
Treatment		**	**	**	**	**	**	**	**	**	**
Straw		**	*	**	**	+	NS	NS	**	**	**
Trt. × straw		**	NS	**	**	NS	NS	NS	*	NS	*
SE†		0.3	2.3	0.8	0.2	0.2	0.1	2.6	1	1	1

NS, +, *, **, Not significant, and significant at the 0.1, 0.05, and 0.01 level, respectively.
† Standard error.

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6–12 mg kg^{-1} of ammonium-N remained in the soils treated with fertilizer, out of the 91 mg N kg^{-1} initially added. Amending urea granules with NSGR did not increase the amount of residual ammonium-N found in the soil, over that observed with untreated urea granules, suggesting that the NSGR did not function as a nitrification inhibitor in this trial. In Experiment 2, water was applied only to the bottom of the pot, and the fertilizer granules were placed on the surface. As in Experiment 1, there would have been no water movement through where the fertilizer was applied, so co-retention of the polymer and fertilizer should have been good. Total inorganic N in the untreated urea pots tended to be 3–4 mg N kg^{-1} greater than pots treated with urea + NSGR, suggesting that amending urea with NSGR did not lead to an increase in plant-available N in the soil under application conditions conducive to substantial ammonia loss.

North Dakota Laboratory Experiment 3 Results

This trial was designed to minimize water movement during the initial period of urea hydrolysis and reaction with the soil, which would help keep the urea and Nutrisphere in close contact during incubation. The effect of incubation length, urea, and urea + NSGR on ammonium- and nitrate-N concentrations in the soil is shown in Figure 6. Nitrification of the applied
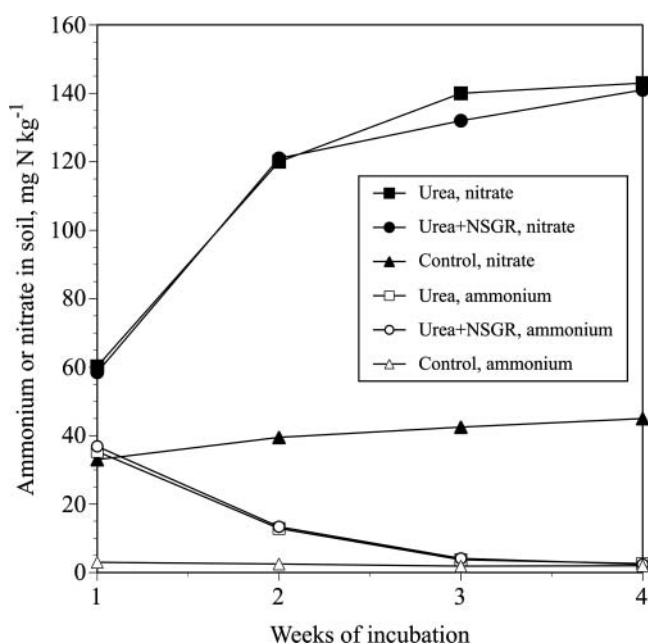

FIGURE 6 Ammonium and nitrate in a Renshaw soil as influenced by length of incubation and application of urea granules, and urea granules treated with Nutrisphere-N for granular fertilizers (NSGR). North Dakota laboratory experiment 3.

TABLE 11 Analysis of variance for rice grain yield as affected by N source (NFS), N timing (NT), N rate (NR), and their interaction for studies conducted at the Delta Research and Extension Center (DREC), Lake Hogue Research Farm (LHRF) and the Rice Research and Extension Center (RREC).

Source	DREC	LHRF	RREC
NFS	NS	NS	NS
NT	*	**	**
NR	**	**	**
NFS × NT	NS	NS	NS
NFS × NR	NS	NS	NS
NFS × NR × NT	NS	NS	NS

NS, *, **; Not significant, and significant at the 0.05, and 0.01 level, respectively.

urea was rapid and largely complete after three weeks. Nitrate concentrations in soil receiving urea and urea+NSGR were nearly identical during the four week incubation (Table 9). Ammonium concentration in soil receiving urea and urea+NSGR was also similar over the duration of the experiment. There was no indication that the treatment of urea granules with NSGR inhibited nitrification.

Mississippi and Arkansas Field Experiment Results

There was no significant ($P < 0.05$) three-way interaction between N source, N rate and N application timing on rice grain yield in any of the three field studies conducted (Table 11). Nor were there any significant ($P < 0.05$) two-way interactions between N source and N rate or N application timing on rice yield in any of the field studies. Significant main treatment effects on rice grain yield did exist for all three field studies for N rate and N application timing, but not N fertilizer source (Table 12).

No precipitation occurred at the DREC during the 10 DBF (days before flooding) after the N sources were applied (Table 5). Rice grain yield increased at the DREC with each incremental increase in N rate applied

TABLE 12 Influence of nitrogen (N) rate, averaged across N sources and application times, on rice grain yield at the Delta Research and Extension Center (DREC), Lake Hogue Research Farm (LHRF) and the Rice Research and Extension Center (RREC)

N rate (kg N ha^{-1})	Grain yield (Mg ha^{-1})		
	DREC	LHRF	RREC
0	4.84	7.00	2.82
67	—	8.11	6.15
101	7.91	—	—
134	—	8.58	8.01
168	9.17	—	—
LSD (0.05)	0.35	0.40	0.50

TABLE 13 Influence of nitrogen (N) fertilizer application timing, averaged across N sources and rates, on rice grain yield at the Delta Research and Extension Center (DREC), Lake Hogue Research Farm (LHRF) and the Rice Research and Extension Center (RREC)

	Grain yield (Mg ha^{-1})		
N Timing DBF[†]	DREC	LHRF	RREC
1	8.77	8.92	8.27
5 or 6	—	8.22	7.26
10 or 11	8.32	7.86	5.75
LSD(0.05)	0.35	0.40	0.50

[†]The number of days before flood (DBF) was 1 and 10 DBF for DREC, 1, 5, and 11 DBF for LHRF, and 1, 6, and 10 DBF for RREC.

which indicates there was a response to N fertilizer at this site (Table 13). In addition, there was a significant grain yield decrease at the DREC when the N sources were applied 10 DBF compared to 1 DBF (Table 14). However, urea + 0.25% and 0.05% NSN resulted in no grain yield increase compared to urea, averaged across N application times (Table 15).

Only a trace of precipitation occurred between any of the N application times and flooding at the LHRF (Table 5). Similar to the DREC, rice grain yield at the LHRF increased significantly as N rate increased (Table 13). Somewhat similarly, rice grain yield at the LHRF displayed a significant decrease when the time between N application and flooding was increased from 1 to 5 and 1 to 11 DBF, but not when increased from 5 to 11 DBF (Table 14). Rice grain yield was not significantly different between urea + 0.25% NSN and urea (Table 15) and there was no interaction between N source and N application timing at the LHRF (Table 12). This suggests enough N fertilizer loss occurred, probably mostly via ammonia volatilization, between 1 and 5 or 11 DBF at the LHRF to cause rice grain yield to decrease and NSN was unable to reduce this N loss enough to effect rice yield (Table 14).

TABLE 14 Influence of nitrogen (N) fertilizer source on rice grain yield at the Delta Research and Extension Center (DREC), Lake Hogue Research Farm (LHRF) and the Rice Research and Extension Center (RREC)

	Grain yield (Mg ha^{-1})		
N source	DREC	LHRF	RREC
Urea + 0.25% NSN[†]	8.52	8.27	7.00
Urea + 0.05% NSN[†]	8.42	—	—
Urea	8.67	8.42	7.16
LSD (0.05)	NS[‡]	NS	NS

[†]NSN = Nutrisphere-N.

[‡]NS = not significant at the 0.05 probability level.

TABLE 15 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.

N sources	Days after N source application			
	3	7	11	15
	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.

    The weather necessary to quantify the influence of NSN-N on ammonia volatilization and the resulting rice grain yields was not as conducive at the RREC location as the other two locations because >5 cm of precipitation occurred the day after the 10 DBF N application, which should have suppressed ammonia volatilization from urea (Table 5). However, the 10 DBF N application at the RREC can be used to evaluate the nitrification inhibition abilities of NSN and the 6 DBF N application can be used to evaluate ammonia volatilization inhibition. Grain yield increased incrementally with increasing N rate at RREC, mirroring the yield results at DREC and LHRF (Table 13), decreased when the flood was delayed from 1 to 6 DBF and 6 to 10 DBF (Table 14), and was unaffected by NSN addition. Results suggest there was substantial ammonia volatilization loss between 1 and 6 DBF and NSN did not minimize this loss enough to affect rice grain yield. Also, NSN is probably not an effective nitrification inhibitor because yield decreased between the 10 and 6 or 1 DBF N application times at the RREC. The 10 DBF N application at the RREC received 5.1 cm of precipitation the next day that would have effectively incorporated the N fertilizer and halted ammonia volatilization (Savin et al., 2007). Thus, nitrification during the remaining 9 days before flood establishment followed by denitrification after flooding would have been the primary N loss mechanism involved in N treatments applied 10 DBF.

    The significant yield decrease when the flood was delayed from 1 to 5 or 6 and/or 10 or 11 days after N application at the three sites suggests there was substantial enough N loss via ammonia volatilization, nitrification, or both during the period before flooding to significantly decrease grain yield. The aforementioned results coupled with the lack of a significant interaction

between N source and N application timing on grain yield suggests NSN-N did not influence ammonia volatilization enough to minimize the grain yield decrease when applied at 5 or 6 and/or 10 or 11 DBF. Ammonium sulfate has been shown to have significantly less ammonia volatilization loss compared to urea when the flood was delayed for an extended period after N application and to result in greater N uptake and grain yield of rice (Griggs et al., 2007). Additionally, urea + NBPT has been shown to be a superior fertilizer compared to untreated urea when applied between 5 or 10 DBF as concerns ammonia volatilization loss, and N uptake and grain yield of rice (Norman et al. 2006, 2009).

Arkansas Laboratory Study Results

The Arkansas laboratory study measured the cumulative ammonia volatilization of urea + 0.25% NSN in comparison to untreated urea, ammonium sulfate, and urea + NBPT. Cumulative ammonia volatilization was affected by the N source $\times$ sample time interaction ($P = 0.0001$). Ammonium sulfate lost the least amount of N via ammonia volatilization over the 15-day incubation (Table 15). Urea + NBPT displayed similarly small amounts of ammonia volatilization at 3 and 7 days after application, but significantly greater loss than ammonium sulfate at the 11- and 15-day sampling times. The urea + NBPT lost significantly less N via ammonia volatilization at each of the sampling times compared to urea and urea + 0.25% NSN. The ammonia volatilization loss of urea at each sampling time was mirrored closely by the ammonia volatilization loss of urea + 0.25% NSN. Consequently, NSN had no significant effect on ammonia volatilization loss of urea, which explains the lack of grain yield response to NSN in the field.

SUMMARY

Field experiments on spring wheat and durum wheat in North Dakota and field experiments on rice in Mississippi and Arkansas showed no response to urea + NSN compared to similar rates of untreated urea. Laboratory-incubation experiments in North Dakota and Arkansas showed no nitrification inhibiting properties for NSN nor did they show NSN inhibiting ammonia volatilization of urea. At the rates recommended by the manufacturer, NSN was not effective in improving crop yield nor did it perform the claims of the manufacturer (Specialty Fertilizer Products, LLC, Leawood, KS, USA) in laboratory experiments. Growers would be well advised to use products to delay nitrification and urea volatilization that have been shown effective in previous research.

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