A Comparison of a Maleic-Itaconic Polymer and *N*-(*n*-butyl) Thiophosphoric Triamide as Urease Inhibitors

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A maleic-itaconic polymer (MIP) is claimed to inactivate soil urease by nickel removal from the enzyme. Four studies were conducted to compare the urease inhibition properties of two commercial formulations of MIP with a commercial formulation of *N*-(*n*-butyl) thiophosphoric triamide (NBPT). In the first study, addition of MIP at 1, 5, or 50 mg kg^{-1} provided 0 to 2% inhibition of urea hydrolysis, averaged across three soils. At 500 mg kg^{-1}, MIP inhibited urea hydrolysis by 7 to 9%, averaged across three soils. By contrast, addition of NBPT at 1 or 5 mg kg^{-1} inhibited urea hydrolysis by an average of 64 and 72%, respectively. In a second study, 13 carboxylic acids of known nickel sequestration properties, two MIP formulations, and NBPT were evaluated as soil urease inhibitors. Averaged across three soils, MIP and the other carboxylic acids provided <5% inhibition of urea hydrolysis when applied at 50 mg kg^{-1}. Addition of NBPT at 1 and 5 mg kg^{-1} provided an average of 62 and 72% inhibition. In two additional studies using purified jackbean [*Canavalia ensiformis* (L.) DC.] urease in the absence of soil, NBPT provided complete inhibition at a concentration of 1 mg L^{-1} in the reaction mixture. Adding MIP at rates up to 100 mg L^{-1} either did not inhibit urease, or stimulated urea hydrolysis. It was concluded that MIP was not an effective inhibitor of soil or jackbean urease. It was also concluded that nickel sequestration by carboxylic acids is an unlikely mode of action for soil urease inhibition.

Abbreviations: CDTA, cyclohexane-1,2-diaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; HEDTA, hydroxyethylenediaminetriacetic acid; MIP, maleic-itaconic polymer; NBPO, *N*-(*n*-butyl) phosphoric triamide; NBPT, *N*-(*n*-butyl) thiophosphoric triamide; NSN-1, Nutrisphere-N for granular urea; NSN-2, Nutrisphere-N Quick Dry for granular urea; NTA, nitrilotriacetic acid.

Urea fertilizer is subject to loss by ammonia volatilization when applied to soil surfaces without incorporation. The soil, environmental, and management factors influencing the degree of ammonia loss have been summarized by Hargrove (1988). Reducing ammonia loss by amending the fertilizer with a urease inhibitor is an accepted agronomic practice (for recent reviews, see Chen et al., 2008; Chien et al., 2009; Watson et al., 2009). Kiss and Simihaian (2002) estimated that more than 14,000 compounds or mixtures of compounds have been evaluated for their effect on soil urease. The compound *N*-(*n*-butyl) thiophosphoric triamide (NBPT) is the most widely-used soil urease inhibitor (Watson et al., 2009). The effectiveness of NBPT as a soil urease inhibitor and its reactions in the soil are well documented (Bremner et al., 1991; Bremner and Chai, 1986; Creason et al., 1990; Hendrickson and Douglass, 1993; McCarty et

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al., 1989). Medina and Radel (1988) reviewed the modes of action of urease inhibitors, and categorized NBPT as a structural analog of urea.

In recent years, a new product has been widely promoted and sold in the United States as a soil urease inhibitor. The product is a polymer of two carboxylic acids, a MIP sold as Nutrisphere-N (SFP, Leawood, KS). It is claimed that this polymer "...pulls the nickel out of the urease molecule, destabilizing the molecule rendering it ineffective..." (Sanders, 2007). Although no research papers establishing this mode of action for soil urease inhibition have been found, the concept has been widely promoted to farmers and fertilizer dealers (for examples, see AgProfessional, 2011; Blaylock and Murphy, 2006 [the name "N-Guard" was used to describe MIP in this article]; Heiniger, 2010; Meece and Pewitt, 2011; Tindall 2008; United Suppliers, 2012). No papers demonstrating that MIP inhibits soil urease have been found. A laboratory study showed no effect of MIP on the rate of urea hydrolysis when included in droplets of urea solutions applied to soil (Franzen et al., 2011).

The objectives of this research were to compare the effectiveness of two formulations of MIP with a commercial source of NBPT as soil urease inhibitors, to evaluate nickel sequestration as a possible mode of action of inhibition of soil urease, and to compare the effects of MIP and NBPT on purified jackbean urease.

MATERIALS AND METHODS

General

The source of NBPT was Agrotain Ultra, donated by Koch Agronomic Services, Wichita, KS. The material was used according to the analysis on the label, 26.7% NBPT by weight. The two sources of MIP were Nutrisphere-N for granular urea (NSN-1) donated by the J.R. Simplot Company, Boise, ID, and Nutrisphere-N Quick Dry for granular urea (NSN-2), obtained from Arthur Farmers Elevator, Arthur, ND. Both Nutrisphere-N products were used assuming an analysis of 40% MIP by weight, the minimum analysis listed on the label.

Table 1. Soil properties.

Property†	Units	Renshaw	Glyndon	Nicollet
pH		7.3	8.1	6.5
EC	dS m^{-1}	0.4	0.5	0.6
$CaCO_3$	g kg^{-1}	0	29	0
CEC	cmol(+) kg^{-1}	7.1	18.6	27.8
Sand	g kg^{-1}	675	650	225
Silt	g kg^{-1}	125	100	325
Clay	g kg^{-1}	200	250	450
Texture		sandy loam	sandy clay loam	clay
Organic matter	g kg^{-1}	18	28	59
Olsen P	mg kg^{-1}	2	11	28
Exch. K	mg kg^{-1}	80	60	275

† pH and electrical conductivity (EC) on a 1:1 soil/water suspension, $CaCO_3$ by pressure calcimetry, cation exchange capacity (CEC) by the sodium acetate method, texture by the hydrometer method, organic matter by weight loss on ignition, available P by the Olsen test, and available K by ammonium acetate extraction.

Four incubation studies were conducted under laboratory conditions. Two studies used A horizon samples of Renshaw (Calcic Hapludolls), Glyndon (Aeric Calciaquolls), and Nicollet (provided by Jerry Hatfield, USDA-ARS, Ames, IA) (Aquic Hapludolls) soils. The soils were collected in the field, air-dried, crushed, and sieved to pass a 2-mm sieve. The properties of the soils used are listed in Table 1. Two studies utilized purified jackbean urease solutions in the absence of soil. All four incubation studies were performed at 25°C, and each study had three replications. Statistical analysis included ANOVA and calculation of the LSD at the 0.05 level for comparison of treatment means.

Inhibitor Rate Study

The inhibitor rate study compared rates of MIP and NBPT on the rate of urea hydrolysis by three soils. One-half milliliter aliquots of water (no inhibitor control) or a test inhibitor solution were placed into 25 mL plastic cups. The test inhibitor solutions contained, per 0.5 mL, 10, 50, 500, or 5000 μg of MIP as NSN-1, 10, 50, 500, or 5000 μg of MIP as NSN-2, or 10 or 50 μg of NBPT. Next, 0.5 mL of a solution containing 5 mg of urea was placed in each cup. Additional water was added, according to soil texture, 0 mL for Renshaw, 1 mL for Glyndon, and 2 mL for Nicollet, to bring each soil to near field capacity. The liquid in each cup was mixed, and 10 g of the appropriate soil added. A plastic lid with a 1 mm hole for aeration was affixed to each cup, and the cups placed in a constant temperature chamber. After 12 h of incubation, the cups were placed in a freezer (−20°C) for 1 to 2 d until the soil could be extracted. The samples were removed from the freezer by replicate, the soil extracted with 100 mL of 0.01 M $CaCl_2$ for 5 min, and the suspensions filtered. A 0.3-mL aliquot of the filtrate was analyzed for urea by the method described below.

Percent inhibition of urea hydrolysis was calculated after Bremner and Chai (1986) as $100 \times [(U\text{-}C) - (U\text{-}I)] \times (U\text{-}C)^{-1}$, where U = the amount of urea originally added, 5 mg, C = mg of urea remaining after incubation in the control, and I = mg of urea remaining after incubation in the presence of a test inhibitor.

Nickel Sequestration Study

The same three soils as used in the first study were treated with carboxylic acids with a wide range of stability constants for nickel (Table 2). The test inhibitor solutions were prepared as follows. One gram of itaconic, maleic, malic, oxalic, citric, salicylic, or imidodiacetic acid was dissolved in water and made to 1 L. The other materials, nitrilotriacetic acid (NTA), ethylene glycol tetraacetic acid (EGTA), hydroxyethylenediaminetriacetic acid (HEDTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), cyclohexane-1,2-diaminetetraacetic acid (CDTA), were inadequately soluble in the free acid form, so 1 g of each of these materials was suspended in 500 mL of water, adjusted to pH 7 with dilute NaOH to affect dissolution, and then brought to 1 L. Solutions containing 1 g L^{-1}

of MIP (2.5 g L^{-1} of NSN-1 or NSN-2) were prepared without pH adjustment. Also, solutions containing 20 and 100 mg L^{-1} of NBPT were prepared without pH adjustment. One-half milliliter aliquots of water (no inhibitor control), or test inhibitor solution, 0.5 mL of urea solution, and additional water by soil type were added to 25 mL plastic cups, as in the prior experiment. After mixing, 10 g of the appropriate soil were added, lids affixed, and placed in a constant temperature chamber as in the prior experiment. After 12 h the samples were frozen, and extracted and analyzed as in the prior experiment.

Purified Urease Time-Course Study

Five milliliters of buffer, 5 mL of a urease solution, and 5 mL of water (no inhibitor control) or a test inhibitor solution were placed into 50 mL plastic centrifuge tubes. The buffer was 0.2 M tris(hydroxymethyl)aminomethane, adjusted to pH 7 with HCl before making to volume. The urease solution was 1 g L^{-1} of jackbean urease (Sigma catalog no. 94280). The test inhibitor solutions were 40 mg L^{-1} of MIP as NSN-1, 40 mg L^{-1} of MIP as NSN-2, or 4 mg L^{-1} of NBPT. The tubes were capped, placed on a shaker, and the inhibitors allowed to react with the enzyme for 1 h. Then 5 mL of substrate solution, 200 mg urea L^{-1}, were added to each tube, followed by mixing. After 5 min, 0.3-mL aliquots were taken from each tube, and analyzed for urea as described below. The tubes were resealed, placed on a shaker, and continuously agitated. The tubes were periodically removed from the shaker to allow for additional samples to be taken 30, 60, 90, and 120 min after addition of the urea substrate.

Purified Urease Inhibitor Concentration Study

The test inhibitor solutions were prepared as follows. One gram of NSN-1 or NSN-2 was diluted to 500 mL, brought to pH 7 with 0.05 M NaOH, and diluted to 1 L. This represented 400 mg L^{-1} of MIP. This solution was subjected to 10-fold serial dilution to produce solutions of 40, 4, 0.4, and 0.04 mg L^{-1} of MIP. A solution representing 400 mg L^{-1} of NBPT was prepared. The pH of this solution was 7.08, so its pH was not adjusted. This solution was diluted to prepare solutions of 0.04, 0.4, or 4 mg L^{-1} of NBPT. As in the prior study 5 mL of buffer, 5 mL of urease solution, and 5 mL of water or test inhibitor solution were placed in centrifuge tubes and shaken for 1 h, followed by addition of 5 mL of a substrate solution containing 200 mg urea L^{-1}. The tubes were sealed, and placed on a shaker. After 120 min, a 0.3-mL aliquot of the reaction mixture was taken from each centrifuge tube and analyzed for urea content.

Urea Analysis

The method was adapted from Greenan et al. (1995). In all studies, a 0.3-mL aliquot of soil extract or reaction mixture was mixed with 7 mL of color developing reagent in a screw-top glass culture tube. After sealing and mixing, the test tubes were placed in a 90°C water bath in the dark for 1 h, followed by rapid cooling to room temperature. Absorbance was measured at 525 nm. The color developing reagent contained 100 mL of water, 100 mL of a mixed acid reagent, 6.2 mL of a diacetyl monoxime solution, and 3.9 mL of a thiosemicarbazide solution. The mixed acid reagent consisted of 960 mL of phosphoric acid and 40 mL of sulfuric acid. The diacetyl monoxime solution consisted of 3.75 g dissolved in 100 mL of water. The thiosemicarbazide solution consisted of 0.375 g dissolved in 100 mL of water.

RESULTS AND DISCUSSION

Inhibitor Rate Study

The effect the rate of MIP and NBPT on the amount of urea remaining in three soils is shown in Table 3. The application of 1, 5, or 50 mg kg^{-1} of MIP as NSN-1 or NSN-2 product provided <5% inhibition of urea hydrolysis by the three soils used in this study. There was a weak (16–17%) inhibition of urea hydrolysis when the two MIP formulations were applied to the Renshaw sandy loam at 500 mg kg^{-1}, but this rate (equal to the rate of urea applied) provided ≤5% inhibition with the other two soils. By contrast, addition of NBPT provided substantial inhibition of urea hydrolysis of these three soils, averaging 64 and 72% at 1 and 5 mg kg^{-1}, respectively. The performance of NBPT in this study was similar to what was obtained by Bremner and Chai (1986), who observed an average inhibition of urea hydrolysis of 67 and 79% when 1 or 5 mg kg^{-1} of NBPT was incubated with six Iowa soils for 2 d.

Urease inhibitors intended for surface impregnation on granular urea must be active at very low concentrations in the soil. For example, the rate of NBPT or MIP commercially applied to fertilizer is approximately 1 kg of active ingredient Mg^{-1} of granular urea. If a 10 mg urea pellet coated with 10 μg of the active ingredient of an inhibitor reacts with 10 g of soil in the field, the concentration of the inhibitor in the soil reaction zone would be approximately 1 mg kg^{-1}. Application of 1 mg kg^{-1} of NBPT significantly inhibited urea hydrolysis (64% in this study),

Table 2. Thirteen carboxylic acids tested for soil urease inhibition properties, and their stability constants for sequestration of Ni^{2+}.

Material	Stability constant†	Reference
	log K‡	
Itaconic acid	1.8	Martell and Smith, 1977
Maleic acid	2.0	Martell and Smith, 1977
Malic acid	3.17	Martell and Smith, 1977
Oxalic acid	5.3	Sillen and Martell, 1964
Citric acid	5.40	Martell and Smith, 1977
Salicylic acid	6.95	Smith and Martell, 1989
Imidodiacetic acid	8.13	Martell and Smith, 1974
NTA	11.50	Martell and Smith, 1974
EGTA	13.50	Martell and Smith, 1974
HEDTA	17.1	Martell and Smith, 1974
EDTA	18.52	Martell and Smith, 1974
DTPA	20.17	Martell and Smith, 1974
CDTA	20.2	Martell and Smith, 1974

† Temperature = 25°C, ionic strength 0.1. See references for explanation.

‡ $K = [NiL] \times [Ni]^{-1} \times [L]^{-1}$, where [Ni] = the concentration of the uncomplexed Ni^{2+}, [L] = the concentration of unbound ligand, and [NiL] = the concentration of nickel-ligand complex.

Table 3. Amount of urea remaining after incubation with three soils and percent inhibition of urea hydrolysis, as influenced by three products sold as soil urease inhibitors. Initial urea application, 5 mg.

Inhibitor	Rate	Renshaw		Glyndon		Nicollet		Average inhibition
		Urea remaining	Inhibition	Urea remaining	Inhibition	Urea remaining	Inhibition	
	mg kg^{-1}	mg	%	mg	%	mg	—— % ——	
None	–	1.52		1.26		1.33		
NSN-1	1	1.50	–1	1.33	2	1.39	2	1
	5	1.54	1	1.33	2	1.46	4	2
	50	1.56	1	1.40	4	1.40	2	2
	500	2.12	17	1.44	5	1.53	5	9
NSN-2	1	1.40	–3	1.40	4	1.38	1	1
	5	1.47	–1	1.33	2	1.36	1	1
	50	1.47	–1	1.32	2	1.33	0	0
	500	2.06	16	1.31	1	1.46	4	7
NBPT	1	3.93	69	3.39	57	3.79	67	64
	5	4.19	77	3.57	62	4.11	76	72
LSD(0.05)		0.10		0.09		0.07		

but MIP was ineffective at 50 mg kg^{-1} for all three soils, and a weak inhibition was only observed with one soil at 500 mg kg^{-1}. These results agree with Franzen et al. (2011) where no effect of MIP on urea hydrolysis was observed with two soils treated with small droplets of urea solutions. These results are also consistent with other studies (Connell et al., 2011; Franzen et al., 2011; Goos, 2013) where it was observed that NBPT was much more effective than MIP at reducing ammonia loss from surface-applied urea granules or urea-ammonium nitrate liquid.

Nickel Sequestration Study

The effect of 13 carboxylic acids, two MIP products, and NBPT on urea hydrolysis is shown in Table 4. The rate of urea hydrolysis by the Glyndon and Nicollet soils was not significantly slowed by adding any of the carboxylic acids, including MIP, at a rate of 50 mg kg^{-1}. With the sandy Renshaw soil, addition of 50 mg kg^{-1} of maleic acid provided 10% inhibition of urea hydrolysis, and itaconic, malic, oxalic, and citric acids provided 5 to 6% inhibition. All of the higher molecular weight chelating agents with a substantial ability to sequester nickel (NTA, EGTA, HEDTA, EDTA, DTPA, CDTA) were completely ineffective at inhibiting soil urease. By contrast, the addition of NBPT provided 62 and 72% inhibition of urease at 1 and 5 mg kg^{-1}, respectively, when averaged across the three soils. The observation that carboxylic acids are ineffective as soil urease inhibitors is in agreement with Sahrawat (1979) who found that tartaric acid, citric acid, and oxalic acid, NTA and EDTA provided <5% inhibition of soil urease activity when applied at 50 mg kg^{-1}. Cai et al. (1989) demonstrated that EDTA was a very weak inhibitor of soil urease activity. Saturating the soil with 2 mM EDTA (almost

Table 4. Effect of 13 carboxylic acids and three products sold as soil urease inhibitors on urea remaining after 12 h of incubation with soil. Original urea application was 5 mg.

Test inhibitor	Inhibitor concentration	Renshaw		Glyndon		Nicollet		Average inhibition
		Amount	Inhibition	Amount	Inhibition	Amount	Inhibition	
	mg kg^{-1}	mg	%	mg	%	mg	—— % ——	
None	–	1.18	–	0.77	–	1.25	–	–
Itaconic acid	50	1.39	5	0.81	1	1.30	1	2
Maleic acid	50	1.58	10	0.75	0	1.35	3	4
Malic acid	50	1.38	5	0.68	–2	1.25	0	1
Oxalic acid	50	1.41	6	0.78	0	1.20	–1	2
Citric acid	50	1.42	6	0.74	–1	1.21	–1	1
Salicylic acid	50	1.24	2	0.72	–1	1.39	4	2
Imidodiacetic acid	50	1.34	4	0.77	0	1.30	1	2
NTA	50	1.25	2	0.77	0	1.30	1	1
EGTA	50	1.26	2	0.76	0	1.22	–1	0
HEDTA	50	1.20	1	0.72	–1	1.14	–3	–1
EDTA	50	1.19	0	0.71	–1	1.21	–1	–1
DTPA	50	1.19	0	0.78	0	1.24	0	0
CDTA	50	1.13	–1	0.77	0	1.21	–1	–1
NSN-1	50	1.17	0	0.77	0	1.25	0	0
NSN-2	50	1.17	0	0.76	0	1.19	–2	–1
NBPT	1	3.68	65	3.20	57	3.69	65	62
NBPT	5	4.03	75	3.54	65	4.08	75	72
LSD(0.05)		0.17		0.12		0.16		

600 mg EDTA L^{-1}) provided a 39.6% inhibition of urea hydrolysis after 2 d, and 4.2% inhibition after 4 d.

The claimed mode of action for MIP is that the polymer of two carboxylic acids removes nickel from the urease enzyme (Sanders, 2007). This mode of action was not demonstrated in this study, despite the fact that the experiment included very strong chelating agents for nickel. Sommers and Lindsay (1979) conducted simulations of the preference of chelating agents for metals in soil environments. So effective were EDTA, HEDTA, DTPA, and CDTA at sequestering nickel that they predicted near 100% saturation of these ligands with nickel in most of their simulations. They stated, "In essence, EDTA, CDTA, DTPA, and HEDTA should extract most of the labile Ni from soils." If very strong chelating agents for nickel did not inhibit urease, then nickel sequestration by carboxylic acids is an unlikely mode of action for the inhibition of soil urease.

Purified Urease Time-Course Study

The effect of MIP and NBPT on the rate of urea hydrolysis by jackbean urease at pH 7 is shown in Fig. 1. A concentration of 1 mg L^{-1} of NPBT in the reaction mixture completely inhibited jackbean urease. This result does not agree with the findings of McCarty et al. (1989), who found little effect of NBPT on jackbean and bacterial urease at a concentration of 1 mg L^{-1}. It is known that NBPT, per se, is a weak urease inhibitor, and must be converted to *N*-(*n*-butyl) phosphoric triamide (NBPO) to be fully active (Creason et al., 1990; Hendrickson and Douglass, 1993). It is possible that the commercial NBPT product used contained NBPO, or related compounds that inhibit urease. Alternatively, it is possible that some NBPO formed during the shaking process before the urea was added, or there was some reaction between NBPT and the urease enzyme during the 1 h shaking before addition of urea. Davies and Shih (1984) demonstrated that several urease inhibitors were more effective if reacted with the enzyme before adding urea. For example, they showed that 1 mM L-aspartyl-4-hydroxamate did not inhibit plant-derived urease when added simultaneously with urea, but provided 85 to 88% inhibition when reacted with the enzyme for 1 h before adding urea.

There was no inhibition of urease by MIP in this study. A concentration of 10 mg L^{-1} of MIP in the reaction mixture stimulated urea hydrolysis, compared to the no-inhibitor control (Fig. 1). The reason for this stimulation is not known.

Purified Urease Inhibitor Concentration Study

The effect of inhibitor concentration on urea hydrolysis by purified jackbean urease is shown in Fig. 2. The inclusion of NSN-1 or NSN-2 in the reaction mixture had no inhibitory effect on urease, and at higher rates stimulated the activity of the enzyme. For example, the highest concentration of MIP tested, 100 mg L^{-1}, was twice the concentration of the substrate and was allowed to react with the enzyme for 1 h before urea addition. Addition of MIP at this concentration stimulated urea hydrolysis. By contrast, inclusion of 0.1 and 1 mg L^{-1} of NBPT in the reaction mixture substantially or completely inhibited urease, despite the limited time for the active oxon NBPO to form.

The commercial MIP product Nutrisphere-N is widely sold in the United States as a soil urease inhibitor. No research papers have been found that demonstrate that MIP functions as a soil urease inhibitor. This paper and Franzen et al. (2011) have shown that the product has little ability to inhibit soil urease. It has also been shown (Connell et al., 2011; Franzen et al., 2011; Goos, 2013) that MIP is considerably less effective than NBPT in reducing ammonia volatilization from surface applications of granular urea or liquid urea-ammonium nitrate fertilizer.

The claimed mode of action of MIP, namely, nickel removal from the urease enzyme by sequestration, does not seem to be a likely mode of action for soil urease inhibition. It has been shown here and by Sahrawat (1979) that carboxylic acids and chelating agents with strong affinity for nickel have little or

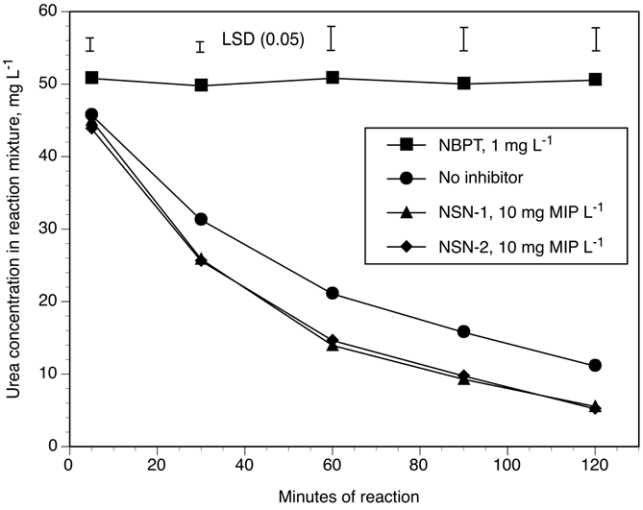

Fig. 1. Effect of three fertilizer additives on urea hydrolysis by jackbean urease at pH 7. NBPT, *N*-(*n*-butyl) thiophosphoric triamide; NSN-1, NSN-2, two sources of maleic-itaconic polymer; MIP, maleic-itaconic polymer.

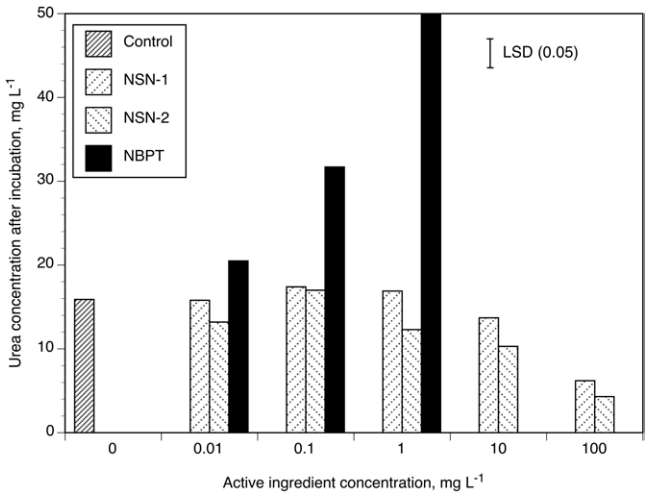

Fig. 2. Effect of inhibitor concentration on urea remaining after 2-h incubation with jackbean urease at pH 7. Initial urea concentration was 50 mg L^{-1}. NBPT, *N*-(*n*-butyl) thiophosphoric triamide; NSN-1, NSN-2, two sources of maleic-itaconic polymer.

no effect on soil urease activity. In presentations given to farmers and fertilizer dealers, the ability of MIP to inhibit soil urease is attributed to its reaction with nickel in the +5 oxidation state (AgProfessional, 2011; Heiniger, 2010; Meece and Pewitt, 2011; Sanders, 2007; United Suppliers, 2012). The most common oxidation state of nickel in geologic materials, soils, and urease is Ni^{2+} (Adriano, 1986; Andrews et al., 1988; Boyle and Robinson, 1988; Coyle and Stiefel, 1988). No reports of the existence of Ni^{5+} in biological systems have been found, and even the existence of Ni^{4+}, "...can probably be ruled out as a biologically viable oxidation state..." (Eidsness et al., 1988). Thus, the claim that Nutrisphere-N inactivates soil urease by reaction with nickel in the +5 oxidation state appears to be implausible.

This paper has also shown that in buffered systems with jackbean urease at pH 7, MIP either had no effect, or actually stimulated urea hydrolysis. It was concluded that MIP was of little value as a urease inhibitor under the conditions of these four studies, and the proposed mode of action of the product is doubtful.

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