Duromide increase NBPT efficiency in reducing ammonia volatilization loss from urea

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ABSTRACT: Novel fertilizer stabilization technologies are needed to decrease ammonia (NH₃-N) losses and increase nitrogen use efficiency. Duromide + NBPT is a new technology that combines two molecules, Duromide and NBPT, whose propose is to increase the efficiency of the urease inhibitor NBPT in reducing NH₃-N loss from urea. Preliminary results showed that Duromide + NBPT can be a more effective product than just NBPT, particularly under low soil pH and high-temperature conditions. This study aimed to compare the effects of urea + Duromide + NBPT, urea + NBPT, and conventional urea on soil N losses by NH₃-N volatilization. The field experiment was conducted on a Latossolo Vermelho Distroférrico (Oxisol) in Campo Mourão, Paraná, Brazil, using a randomized complete block design with treatments arranged in a 2 × 3 factorial, comprising two N doses (45 and 90 kg ha⁻¹) and three N fertilizers (urea + Duromide + NBPT, urea + NBPT, and conventional urea). The NH₃-N volatilization data were subjected to nonlinear regression using a logistic model. The NH₃-N losses varied according to dose and fertilizer, reaching up to 12.4 % of the applied N in the conventional urea treatment. Urea + Duromide + NBPT was more efficient than urea + NBPT in decreasing NH₃-N volatilization. Compared to conventional urea at doses of 45 and 90 kg ha⁻¹ of N, urea + Duromide + NBPT reduced NH₃-N volatilization losses by 35 and 54 % and from urea + NBPT by 15 and 33 %, respectively. The new stabilizing technology Duromide + NBPT reduced NH₃-N losses by up to 33 % compared to NBPT alone. Ammonia volatilization was influenced by soil moisture. The volatilization peak, observed after 18-19 days of N fertilizer application, was triggered by rainfall events.

Keywords: enhanced efficiency fertilizers, nonlinear model, N-stabilizers, urease inhibitor.
INTRODUCTION

Nitrogen (N) is the nutrient required in the greatest amounts and the most limiting factor for plant growth (Souza and Fernandes, 2018). Urea [CO(NH$_2$)$_2$] is the most used N fertilizer worldwide, with a total production of 183.2 Mt yr$^{-1}$, supplying 53.3 % of the current N demand (IFA, 2019).

The popularity of urea as a fertilizer stems from its high N concentration (45 to 46 %), low cost, high solubility, compatibility with most fertilizers, and wide availability (Cantarella et al., 2008; Chien et al., 2009). However, when broadcast onto the soil surface, urea is highly susceptible to losses by leaching, denitrification, and ammonia (NH$_3$-N) volatilization (Gillette et al., 2017). Ammonia volatilization is a major route of N loss from agricultural soils, reaching more than 50 % of N applied (Tasca et al., 2011). Following surface application, urea is hydrolyzed by the action of ureases, resulting in the production of ammonium (NH$_4^+$) and carbon dioxide (CO$_2$), as demonstrated by the reaction CO(NH$_2$)$_2$ + 2H$^+$ + 2H$_2$O $\rightarrow$ 2NH$_4^+$ + H$_2$O + CO$_2$ (Cantarella, 2007). Because urea hydrolysis consumes protons (H$^+$), the reaction results in increased pH around fertilizer granules, shifting the equilibrium toward the formation of NH$_3$-N, which is subsequently lost to the atmosphere in its gaseous form (NH$_3$) (Rochette et al., 2009; Cantarella et al., 2018).

To circumvent these limitations, the fertilizer industry has focused on the development of enhanced efficiency fertilizers (EEFs) (Guelfi, 2017). Perhaps the most studied and used strategy to mitigate NH$_3$-N losses is to combine urea with the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) (Otto et al., 2017; Silva et al., 2017a; Cantarella et al., 2018; Sunderlage and Cook, 2018; Liu et al., 2019). It is estimated that 14 Mt of EEFs, including controlled-release urea, slow-release urea, and fertilizers treated with urease and nitrification inhibitors, were produced in 2016, with urea + NBPT accounting for 7.4 Mt (53 %) of the total EEF production (Cantarella et al., 2018).

For urease inhibition to occur, NBPT must be converted to its oxygen analog (NBPTO) (Guelfi, 2017). Then, NBPTO forms a tridentate bond with the active site of urease, reducing the probability of urea reaching a nickel atom at the enzyme’s active site (Manunza et al., 1999), thereby preventing urea breakdown and reducing NH$_3$-N volatilization. In the early 1980s, Brenner (1995) considered that NBPT was the most promising urease inhibitor to reduce NH$_3$-N losses, even though it was not commercially available at the time.

Meta-analysis studies concluded that NBPT reduce NH$_3$-N losses by 52 to 54 % compared with conventional urea (Pan et al., 2016; Silva et al., 2017a). However, the benefits of NBPT-treated urea do not always translate into yield gains in crop production (Dillon et al., 2012; Prando et al., 2013; Cancellier et al., 2016; Guardia et al., 2017; Yang et al., 2020). Moreover, NBPT does not seem efficient in reducing NH$_3$-N losses under certain conditions (Ribeiro et al., 2020). There are uncertainties about the efficiency of NBPT, because higher temperatures, higher humidity, and the presence of straw in the soil can accelerate the degradation of this molecule, decreasing its efficiency in reducing losses due to NH$_3$-N volatilization (Suter et al., 2011; Engel et al., 2013; Mira et al., 2017). The NBPT efficiency is strongly reduced by values of porous space filled with water above 65 % (Sanz-Cobena et al., 2012). Tasca et al. (2011) observed increase of 12 times in the volatilization with urea treated with NBPT caused by the increase in the ambient temperature from 18 to 35 °C. Another factor that interferes with the efficiency of the NBPT is the soil pH. Soares (2011) observed that NBPT reduced NH$_3$-N volatilization by 52-53 %, compared to urea in soils with pH 5.6 and 6.4. However, the reduction was only 18 % in soil with pH 4.5. Engel et al. (2015) also showed that the NBPT half-life is longer in alkaline soils, with 0.07, 0.59, 2.70, and 3.43 days at pH 5.1, 6.1, 7.6, and 8.2, respectively.

Added to the possible problems of NBPT degradation, beneficial effects on crop yield and N use efficiency are limited, typically ranging from 5 to 12 % (Cantarella et al., 2018). These findings underscore the need for new technologies that improve fertilizer stabilization and effectively reduce NH$_3$-N losses, and provide consistent yield gains.
Duromide + NBPT is a new technology that combines two molecules, Duromide and NBPT, in which preliminary results indicate a more effective product than just NBPT. Duromide is a stabilizer responsible for inhibiting the activity of the urease enzyme, function similar to that found in NBPT molecule alone (Koch, 2020). However, over time, elements such as soil temperature and pH can result in NBPT degradation. The new Duromide stabilizer has a different chemical structure than NBPT, with radicals that make the molecule more stable, allowing more time for N fertilizer to be incorporated into the soil by precipitation or irrigation and consequently reducing N losses by NH₃-N volatilization.

To date, there are no reports on the use of Duromide + NBPT in subtropical soils, as the product has recently entered the market (2020/2021). Our hypothesis is that urea + Duromide + NBPT, followed by urea + NBPT, and conventional urea, respectively, is the most efficient in reducing NH₃-N volatilization losses under field conditions, regardless of the applied dose. This study aimed to compare the effects of urea + Duromide + NBPT, urea + NBPT, and conventional urea on NH₃-N volatilization.

**MATERIALS AND METHODS**

**Experimental design and treatments**

The experiment was conducted in Campo Mourão (23° 98′ 82″ S and 52° 34′ 64″ W), Paraná State, Brazil. The soil of the study site was classified as *Latossolo Vermelho Distroférrico* (Santos et al., 2018), which corresponds to an Oxisol according to the USDA Soil Taxonomy (Soil Survey Staff, 2014). Soil samples were collected at the 0.00–0.20 m soil layer for chemical and physical characterization (Table 1). The climate in the area is classified as Cfa according to Köppen classification system. The data for temperature, precipitation, and relative humidity of the air during the conduction of the field experiment was obtained from INMET (Instituto Nacional de Meteorologia).

A randomized complete block design was used in a 2 × 3 factorial arrangement, with two N doses and three N sources, totaling six treatments and five replications (30 experimental units). Treatments were as follows: conventional urea at 45 and 90 kg ha⁻¹ of N (Ur₄₅ and Ur₉₀), SuperN® known internationally as Agrotain® NBPT treated urea with a concentration 540 mg kg⁻¹ of NBPT at 45 and 90 kg ha⁻¹ of N (Ur₄₅-NBPT and Ur₉₀-NBPT) and SuperN®PRO known internationally as Anvol® Duromide + NBPT treated urea with a concentration 540 mg kg⁻¹ of NBPT and 120 mg kg⁻¹ of Duromide at 45 and 90 kg ha⁻¹ of N (Ur₄₅-Duromide + NBPT and Ur₉₀-Duromide + NBPT). The nitrogen fertilizers were applied by broadcasting at stage V₄ (four fully expanded leaves) at a rate 45 and 90 kg ha⁻¹ on April, 27, 2020, to second-crop corn at no-till system.

**Sampling and determination of ammonia volatilization**

To capture ammonia volatilization, nitrogen fertilizers were weighed separately with an analytical balance and applied manually inside the semi-open static chamber with a total area of 0.007854 m². Immediately after N application, samples were collected on days 1, 2, 3, 7, 10, 14, 16, 20, 23, 26, 29, and 32 days after fertilizer application to quantify NH₃-N volatilization, totaling 12 samples. Briefly, a semi-open static chamber was placed on each plot. Chambers consisted of PET bottles containing a 2.5 cm wide and 25 cm long filter paper strip with its base immersed in a 50 cm³ flask with 20 mL of H₂SO₄ 0.05 mol L⁻¹ and 2 % (v/v) glycerin solution (Araújo et al., 2009). Used chambers were replaced with new ones until ammonia loss stabilized. After each collection, the chambers were rotated between three sites within each plot to minimize the effects of environmental factors, such as rainfall. Subsequently, samples were sent to the Soil Fertility Laboratory of the State University of Maringá, Paraná, Brazil, and refrigerated until analysis. The captured ammonia was determined according to the methodology described by Bower and Holm-Hansen (1980), using UV/VIS spectrophotometry.
**Table 1. Chemical and granulometric analysis of Latossolo Vermelho Distroférrico (Oxisol) and interpretation of values for the surface layer (0.00-0.20 m) according to SBCS/NEPAR (2019)**

| Soil properties | Layer 0.00-0.20 m | Property interpretation |
|-----------------|------------------|-------------------------|
| pH(CaCl₂)       | 5.40             | High                    |
| H + Al (cmol, dm⁻³) | 4.47             | -                       |
| Al³⁺ (cmol, dm⁻³) | 0.00             | Very low                |
| Ca²⁺ (cmol, dm⁻³) | 4.94             | High                    |
| Mg²⁺ (cmol, dm⁻³) | 2.02             | High                    |
| K⁺ (cmol, dm⁻³)  | 0.28             | High                    |
| SB (cmol, dm⁻³)  | 7.24             | -                       |
| CEC₄₅₆ (cmol, dm⁻³) | 11.71           | Medium                  |
| ECEC (cmol, dm⁻³) | 7.24             | High                    |
| BS (%)          | 62               | High                    |
| P (mg dm⁻³)     | 10.40            | High                    |
| S (mg dm⁻³)     | 1.55             | Low                     |
| B (mg dm⁻³)     | 0.19             | Low                     |
| Zn (mg dm⁻³)    | 3.36             | High                    |
| Cu (mg dm⁻³)    | 9.66             | Very high               |
| Fe (mg dm⁻³)    | 32.88            | -                       |
| Mn (mg dm⁻³)    | 155.10           | Very high               |
| OC (g dm⁻³)     | 24.77            | High                    |
| OM (%)          | 4.27             | Very high               |
| Sand (%)        | 22               | -                       |
| Silt (%)        | 7                | -                       |
| Clay (%)        | 71               | -                       |

pH(CaCl₂) (0.01 mol L⁻¹) at a soil:solution ratio of 1:2.5; H+Al was determined by the Shoemaker-McLean-Pratt (SMP) method; Ca²⁺, Mg²⁺, and Al³⁺ extracted with KCl 1 mol L⁻¹; OM: soil organic matter content obtained by organic carbon × 1.724 (Walkley-Black); P, K⁺, Zn, Cu, Fe, and Mn: Mehlich-1 extraction; SO₄²⁻ was extracted by calcium phosphate in acetic acid; B was extracted with hot water; sum of bases (SB): Ca²⁺ + Mg²⁺ + K⁺; CEC: cation exchange capacity at pH 7 (Ca²⁺ + Mg²⁺ + K⁺ + H + Al); ECEC: effective cation exchange capacity (Ca²⁺ + Mg²⁺ + K⁺ + Al³⁺); BS: base saturation [(Ca²⁺ + Mg²⁺ + K⁺)/CEC] × 100; and particle size distribution (sand, silt, and clay) by densimeter method.

**Statistical analysis**

The model selection was processed according to Akaike's information criterion (AIC) (Akaike, 1974), and the models with the smallest AIC were chosen. After to select the model, the data were submitted to nonlinear regression, using the logistic model represented by equation 1, as described by Seber and Wild (2003). The model is traditionally used to estimate cumulative ammonia volatilization (Silva et al., 2017a; Cantarella et al., 2018; Minato et al., 2019, 2020).

\[
\hat{Y} = \frac{\alpha}{1 + \exp\left(-\beta y/v\right)} \quad \text{Eq. 1}
\]

In which \(\hat{Y}\) is the amount of N volatilized in the form of NH₃-N (kg ha⁻¹) at time t; \(\alpha\) is the maximum cumulative volatilization; \(\beta\) is the time at which 50 % of the losses occur, corresponding to the curve inflection point (day on which the maximum daily loss of NH₃-N occurs); t is the time (days); and \(\gamma\) is a parameter of the equation used to calculate the maximum daily loss (MDL) of NH₃-N, as shown in equation 2.

\[
\text{MDL} = \frac{\alpha}{4\gamma} \quad \text{Eq. 2}
\]
RESULTS

The weather conditions and NH$_3$-N volatilization rates during the experimental period are shown in figure 1. Nitrogen fertilizers were applied to dry soil 13 days after a 25 mm rainfall event. The minimum and maximum temperatures during the first 76 h after fertilization (starting on April 27, 2020) were 29.6 and 17.8 °C, respectively, and the relative humidity was lower than the critical relative humidity of urea (75.8 % at 25 °C) (Adams and Merz, 1929). Nitrogen losses through NH$_3$-N volatilization were initiated only after a 12 mm rainfall event (16 days after fertilizer application). Volatilization rates decreased after day 20 to basal emission levels similar to those of the control (Figure 1).

Cumulative NH$_3$-N volatilization followed a sigmoidal pattern, increasing gradually at the beginning of the experiment, reaching the maximum daily loss, and stabilizing after that (Figure 2). The maximum cumulative losses ($\alpha$) of NH$_3$-N, according to the adjusted model, were 11.2, 7.6, 5.1, 4.2, 3.6, and 2.7 kg ha$^{-1}$ in plots treated with Ur$_{90}$, Ur$_{90}$-NBPT, Ur$_{90}$-Duromide + NBPT, Ur$_{45}$, Ur$_{45}$-NBPT, and Ur$_{45}$-Duromide + NBPT, respectively (Table 2). Urea treated with the stabilizers NBPT and Duromide + NBPT reduced NH$_3$-N losses by 14.8 and 35.3 %, respectively, compared with conventional urea at a dose of 45 kg ha$^{-1}$ of N and by 31.9 and 54.2 %, respectively, compared with conventional urea at 90 kg ha$^{-1}$ of N.

The greatest reductions in NH$_3$-N loss were achieved with Ur$_{90}$-Duromide + NBPT (54.2 %) and Ur$_{45}$-Duromide + NBPT (35.3 %) (Table 2). Compared with Ur$_{90}$-NBPT, Ur$_{90}$-Duromide + NBPT reduced NH$_3$-N loss by 32.7 %. Similarly, in comparison with Ur$_{45}$-NBPT, Ur$_{45}$-Duromide + NBPT reduced NH$_3$-N loss by 24.1 %.

Peak NH$_3$-N volatilization ($\beta$) in Ur$_{45}$ and Ur$_{90}$ plots occurred at 18.0 and 18.4 days after fertilizer application, respectively (Table 2). The Ur$_{45}$-NBPT and Ur$_{90}$-NBPT delayed
peak volatilization by 1.3 and 1.2 days, respectively, and Ur45-Duromide + NBPT and Ur90-Duromide + NBPT by 0.8 and 1.1 days, respectively. The Ur90, Ur90-NBPT, Ur90-Duromide + NBPT, Ur45, Ur45-NBPT, and Ur45-Duromide + NBPT treatments afforded maximum daily NH3-N losses of 1.01, 0.89, 0.44, 0.24, 0.31, and 0.20 kg ha\(^{-1}\) day\(^{-1}\) NH3-N, respectively (Table 2). The greatest reductions in the maximum daily loss were observed with Ur90-Duromide + NBPT (66 %) and Ur45-Duromide + NBPT (20 %).

**DISCUSSION**

At the time of fertilizer application, the soil moisture was low, resulting in small N losses by NH3-N volatilization during the first days (Figure 1). This behavior differed from that observed by Minato et al. (2020), who applied N fertilizer to second-crop corn and observed maximum NH3-N volatilization in the first 72 h after application. Such results were attributed to the high soil moisture at the time of application, which occurred 48 h after a rainfall of 60 mm.
The sigmoidal behavior of cumulative NH$_3$-N volatilization is explained by the increase in urease activity (Vale et al., 2014), which is influenced by soil moisture. Under dry soil conditions, the hydrolysis rate of urease is low. However, urease activity increases with soil moisture content up to 20% (Sahrawat, 1984). Above this level, hydrolysis is practically not affected by changes in soil moisture (Cantarella et al., 2018). Nascimento et al. (2013) observed a peak of NH$_3$-N volatilization rates only at 15–16 days after N fertilization, given the lack of soil moisture. It is known that soil moisture influences the peak and duration of NH$_3$-N volatilization. Ribeiro et al. (2020) observed that N losses from amide fertilizers, including conventional urea and NBPT-treated urea, occurred 10 days after application and were associated with increased soil temperature and moisture.

The magnitude of NH$_3$-N loss varies according to the technology used in N fertilizers (Liu et al., 2019; Minato et al., 2020), soil properties (Sunderlage and Cook, 2018), and environmental conditions (Otto et al., 2017), hindering analysis of isolated effects of a given factor on NH$_3$-N loss, especially under field conditions. Ammonia volatilization increased with N dose, regardless of the source. This result was expected because of differences in the amount of reaction substrate (NH$_4^+$) added to the soil. Tasca et al. (2011) reported that NH$_3$-N volatilization increased with an increase in N dose. In contrast, Silva et al. (2017b) observed that cumulative NH$_3$-N volatilization from urea + inhibitor decreased with increasing N doses. According to the authors, the higher reduction in volatilization with the use of urea only can be attributed to saturation of urease active sites as a result of the increase in N dose. With NBPT, saturation of urease active sites was lower, reducing the effect of N dose on N loss.

Another important effect of stabilized fertilizers is the delay in peak volatilization (Table 2). With a delay in initial N loss, there is a greater chance for granules to be incorporated into the soil, through rainfall, for instance, reducing NH$_3$-N volatilization. Dall’Orsoletta et al. (2017) and Ribeiro et al. (2020) observed that NBPT was effective in reducing soil NH$_4^+$ levels in the first days after application compared with conventional urea. In the current study, we found that at 45 kg ha$^{-1}$ of N, Ur-NBPT delayed the volatilization peak by one day compared with Ur. Ur-Duromide + NBPT and Ur-NBPT afforded similar effects at 90 kg ha$^{-1}$ of N, being more efficient than Ur (Table 2). Thus, among the parameters adopted, β was the least affected by the technologies tested. Therefore, future studies should investigate the effect of urea treated with Duromide + NBPT under conditions that potentiate the losses of NH$_3$-N, after application, such as higher soil moisture, high temperature, and the presence of plant residues in the soil.

The delay in NH$_3$-N peak does not always decrease total NH$_3$-N losses; for example, Tasca et al. (2011) observed that the NBPT delayed the peaks of volatilization, which occurred in the first week after application. This means that the parameter β deserves caution in the interpretation and cannot be analyzed in isolation, although higher β values are preferable when considering the gaseous losses of N by enhanced efficiency fertilizers (Besen et al., 2021; Minato et al., 2020).

Both stabilized fertilizers were effective in reducing N losses compared with conventional urea (Table 2). As extensively reported in the literature, NBPT acts by blocking urease activity. Some limitations of NBPT include its short period of effective inhibition and limited lifespan, particularly in acidic soils and hot climates, demonstrating the need for new technology. The agroindustry has been faced with the challenge of improving current urease inhibitors and developing new molecules or mixtures for minimizing N loss (Cantarella et al., 2018). In this scenario, the field results of Ur-Duromide + NBPT are promising. The high effectiveness of the new urease inhibitor Ur-Duromide + NBPT is attributed to the efficiency in inhibiting urease for a longer period, stemming from the high stability of Duromide (Koch, 2020). Sunderlage and Cook (2018) studied the characteristics of 79 soils in the United States of America, aiming to predict NH$_3$-N losses. The authors observed that volatilization increased with decreasing soil pH in soils treated...
with urea and NBPT, indicating that the effectiveness of NBPT is lower in acidic soils, probably because of increased chemical degradation rates. Most of Brazil’s cultivable land is covered by acidic soils, representing an aggravating factor. Soil temperature can also intensify NBPT degradation. This reaction can begin within 2 to 4 days in warm soils (Soares et al., 2012) but may take 10 to 15 days in low-temperature soils, such as those found in temperate climate regions (Watson et al., 2008).

Therefore, the efficiency of new technologies, such as Duromide + NBPT, must be investigated under different conditions, on soils of different physical, chemical, and biological characteristics, as well as in the most varied crop systems. Both inhibitors were effective in reducing NH$_3$-N losses, but Duromide + NBPT was more efficient in mitigating N loss than NBPT alone. Although the present study shows the potential of the new technology Duromide + NBPT to reduce NH$_3$-N volatilization more efficiently when compared to NBPT alone, future studies are needed to verify the benefits of the Duromide molecule added to the NBPT in increasing crop yields. Furthermore, our results show that logistic models are an important tool to evaluate N fertilization efficiency. Nitrogen fertilizer stabilizers such as Duromide + NBPT, which allow decreasing $\alpha$ and $\gamma$ and increasing $\beta$ values, are desirable, as they can reduce NH$_3$-N emissions to the atmosphere and contribute to N use efficiency.

**CONCLUSIONS**

Ammonia losses varied according to N dose and type of fertilizer, reaching up to 12.4 % of the applied N in the conventional urea treatment.

Ammonia volatilization was influenced by soil moisture, and the peak volatilization occurred 18–19 days after fertilizer application, triggered by rainfall of 12 mm.

Urea with Duromide + NBPT was the most efficient in reducing NH$_3$-N volatilization (35 to 54 % reduction) compared with conventional urea at 45 and 90 kg N ha$^{-1}$, respectively, being more efficient than Ur-NBPT (15 to 33 % reduction).

The new stabilizing technology Duromide + NBPT reduced NH$_3$-N losses by up to 33 % compared to NBPT alone.

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