University of São Paulo "Luiz de Queiroz" College of Agriculture

Increasing NBPT rates to reduce ammonia volatilization losses from urea applied over sugarcane straw

Acácio Bezerra de Mira

Dissertation presented to obtain the degree of Master in Science. Area: Soil and Plant Nutrition

Piracicaba 2016 Acácio Bezerra de Mira Agronomist

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor: Prof. Dr. **RAFAEL OTTO**

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"If you're going to try, go all the way. Otherwise, don't even start. This could mean losing girlfriends, wives, relatives and maybe even your mind. It could mean not eating for three or four days. It could mean freezing on a park bench. It could mean jail. It could mean derision. It could mean mockery, isolation. Isolation is the gift. All the others are a test of your endurance, of how much you really want to do it. And, you'll do it, despite rejection and the worst odds. And it will be better than anything else you can imagine. If you're going to try, go all the way. There is no other feeling like that. You will be alone with the gods, and the nights will flame with fire. You will ride life straight to perfect laughter. It's the only good fight there is."

Charles Bukowski, Factotum

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RESUMO

Aumentando a concentração de NBPT para reduzir as perdas por volatilização de amônia proveniente de ureia aplicada sobre palhada de cana-de-açúcar

A ureia é o principal fertilizante nitrogenado utilizado em todo o mundo, porém, perdas de nitrogênio (N) na forma de amônia (NH₃) são um importante problema associado ao uso desse fertilizante. O tratamento da ureia com N-(n-butil) tiofosfórico triamida (NBPT) reduz a atividade da enzima urease e a volatilização de NH3 em muitos cultivos. Entretanto, a quantidade de palha sobre o solo em sistemas de cultivo da cana-de-açúcar colhida sem queima (CCSQ) afeta a eficiência do tratamento da ureia com NBPT em reduzir as perdas de NH₃. A hipótese deste estudo é que é necessário aumentar a concentração de NBPT na ureia acima da atual concentração comercial (530 mg kg⁻¹) para reduzir as perdas por volatilização, de modo a viabilizar o uso de ureia tratada com NBPT em sistemas de CCSQ. O objetivo desse estudo foi avaliar, em condições de campo, as perdas de NH₃ de ureia tratada com quatro concentrações de NBPT e aplicada sobre a palhada de cana-de-acúcar. Seis experimentos de campo foram conduzidos no Estado de São Paulo, principal área cultivada com cana-de-açúcar no Brasil. Foi utilizado delineamento aleatorizado em blocos com quatro repetições. Os tratamentos consistiram em ureia tratada com NBPT nas concentrações 0, 530, 850, 1500 e 2000 mg kg⁻¹, nitrato de amônio e um tratamento controle (sem adubação nitrogenada). A volatilização e NH3 foi mensurada através de sistema coletor semiestático fechado, contendo dois discos de espuma de polietileno embebidos com solução de ácido ortofosfórico e glicerina. Os discos de espuma foram coletados e substituídos aos 2, 4, 6, 8, 10, 12, 16, 20, 25 e 30 dias após a aplicação dos fertilizantes (DAF). O N retido nas espumas foi extraído usando água deionizada e a concentração de N determinada por Análise por Injeção em Fluxo (FIA). Modelos sigmoides de Boltzmann foram ajustados para as perdas cumulativas de NH3 ao longo dos dias. As médias das perdas acumuladas entre locais foram comparadas usando teste de Tukey e o efeito das concentrações de NBPT foi testado por análise de regressão (P<0.05). Houve forte influência do local e das condições ambientais nas perdas de NH₃. O NBPT foi menos eficiente reduzir as perdas de NH₃ em condições de alta temperatura e grossa camada de palha, provavelmente devido à alta atividade de urease e à degradação prematura do inibidor. O aumento na concentração de NBPT na ureia acima de 530 mg kg⁻¹ não apenas retardou o pico de máxima taxa de perda diária (T_{max}), mas também reduziu as perdas acumuladas de NH₃. As duas maiores concentrações de NBPT promoveram um retardamento médio de seis dias em relação ao Tmax da ureia. Uma redução linear nas emissões de NH₃ foi verificada até a dose de 1000 mg kg⁻¹ de NBPT, que levou a uma redução de 43% nas perdas em comparação à ureia não tratada. Incrementos na concentração de NBPT acima desse valor não se refletiram em redução substancial das perdas de NH₃. Aumentar a concentração de NBPT na ureia demonstrou potencial em reduzir as perdas e NH₃ por volatilização em sistemas de CCSQ, entretanto são necessárias mais pesquisas avaliando o impacto do N preservado no sistema sobre a produtividade da cana-de-açúcar e a viabilidade econômica dessa tecnologia.

Palavras-chave: Perda de nitrogênio; Inibidor de urease; N-(n-butil) tiofosfórico triamida; Fertilizante estabilizado; Aplicação superficial; Cobertura de palhada de cana

ABSTRACT

Increasing NBPT rates to reduce ammonia volatilization losses from urea applied over sugarcane straw

Urea is the main nitrogen (N) fertilizer used worldwide, but N losses in the form of ammonia (NH_3) is a major problem when this fertilizer is topdressed over crop residues. The treatment of urea with N-(n-butyl) thiophosphoric triamide (NBPT) decreases the activity of urease enzyme and volatilization losses in many crops. However, the amount of straw over the soil in green cane trash blanketing (GCTB) systems affect the effectiveness of NBPT-treated urea in reducing NH₃ losses. The hypothesis of this study is that an increase of NBPT concentration in NBPT-treated urea above the commercial concentration adopted nowadays (530 mg kg⁻¹) is necessary to reduce volatilization losses and improve the efficiency of this fertilizer in GCTB systems. The aim of this study was to evaluate, under field conditions, NH₃ losses from urea amended with four NBPT concentrations and applied over sugarcane straw. Six field trials were carried out across the State of São Paulo, the main sugarcane-cropped area in Brazil. It was adopted the randomized block experimental design with four replications. The treatments consisted of urea amended with the NBPT concentrations 0, 530, 850, 1500 and 2000 mg kg⁻¹, ammonium nitrate and a control treatment (without N fertilizer). The NH₃ volatilization was measured through an enclosure semi-static collector system containing two polyethylene foam discs treated with orthophosphoric acid and glycerol. The foam discs were collected and replaced at 2, 4, 6, 8, 10, 12, 16, 20, 25 and 30 days after the fertilizer application (DAF). The N trapped into the foams was extracted using deionized water and the N concentration determined by Flow Injection Analysis (FIA). Boltzmann sigmoidal models were fitted to cumulative losses of NH₃ along the days. Cumulative losses between locals were compared by Tukey HSD and the effect of NBPT concentrations were tested by regression analyses (P < 0.05). There was a significant effect of local and environmental conditions on amount of NH₃ losses. NBPT was less effective in reducing NH₃ losses under high temperatures and thick straw layer, probably because of the high urease activity and the early inhibitor degradation. The increase on NBPT concentration on urea above 530 mg kg⁻¹ not only delayed the time of maximum rate of loss (T_{max}), but also reduced cumulative NH₃ losses. The two higher NBPT concentrations promoted an average delay of six days from untreated urea T_{max}. Linear reduction of NH₃ emissions occurred up to the NBPT concentration of 1000 mg kg⁻¹ that reduced 43% of NH₃ losses as compared to urea. Any increment in NBPT concentration above this range did not reflect in substantial reduction of NH₃ losses. Increase NBPT concentration showed potential in reducing NH₃ volatilization losses under GCTB sugarcane, however, further research is necessary to evaluate the impact of NH₃ savings on sugarcane yield and the economic feasibility of this technology.

Keywords: Nitrogen loss; Urease inhibitor; N-(n-butyl) thiophosphoric triamide; Stabilized fertilizer; Surface application; Green cane trash blanket

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1 INTRODUCTION

Urea is the major nitrogen (N) source used worldwide in agriculture accounting for roughly 56% of the global production and 60% of the Brazilian market of N fertilizers (GARCIA et al., 2011; INTERNATIONAL FERTILIZER INDUSTRY ASSOCIATION, 2013). This is mainly attributed to urea high concentration (450 to 460 g kg⁻¹ N), lower cost per N unit and lower restrictions for purchase and storage, compared to ammonium nitrate and other nitric sources.

However, urea undergoes hydrolysis resulting in N volatilization loss as ammonia (NH_3) , which reduces efficiency and consequently the economic advantage of this fertilizer (LARA CABEZAS; KORNDORFER; MOTTA, 1997). Hydrolysis of urea is promoted by the enzyme urease, resulting in increase of pH surrounding the granule, promoting the conversion of ammonium (NH_4^+) into NH_3 even in acidic soils (MIKKELSEN, 2009; TASCA et al., 2011). Urease is naturally found in the soil, mostly synthesized by soil microorganisms, although plant residues may supply urease directly to the soil (DHARMAKEERTHI; THENABADU, 1996).

The formation and loss of NH₃ increase when urea is applied superficially, without incorporation, under warm temperatures and moist soil conditions (BOUWMEESTER; VLEK; STUMPE, 1985; TASCA et al., 2011). This hinders the buffering through the exchangeable soil acidity and the deep diffusion and adsorption of NH₄⁺ in negative charges in the soil, subjecting the ion to a high pH area surrounding the granule. The high pH leads to the formation of NH₃ that, due to the proximity to the soil surface, is easily carried to the atmosphere through soil water evaporation and displaced by the wind. In addition, in systems where urea is surface applied, plant residues on the soil surface favor the volatilization by hindering the contact between the soil and the fertilizer, and promoting urease activity far higher than in bare soil (BARRETO; WESTERMAN, 1989).

These NH₃ losses from urea have become a major issue in sugarcane crop systems in Brazil since the burning of sugarcane fields prior harvesting was restricted in 2014. Besides, the ratoon N fertilization started to be superficially applied over a straw layer left on the soil surface after harvesting, ranging from 10 to 20 Mg ha⁻¹ (VITTI et al., 2007). Losses of NH₃ from application of urea over crop residues in the green cane trash blanketing systems (GCTB) range from 11% to 36% and average 25% under Brazilian field conditions (COSTA; VITTI; CANTARELLA, 2003; VITTI et al., 2007; CANTARELLA et al., 2008).

Sugarcane fields receive from 60 to 120 kg ha⁻¹ of N annually (SPIRONELLO et al., 1997) and, with a planted area of 9 million hectares, accounts for 23% of nitrogen fertilizer

consumption in Brazilian agriculture (CANTARELLA; ROSSETTO, 2012). Thereby, although NH₃ losses represents a serious economic issue to the sugarcane growers by reducing the nutritional value of the applied urea (LARA CABEZAS; KORNDORFER; MOTTA, 1997), NH₃ lost can equally impact the environment (BOUWMAN et al., 1997; TURNER et al., 2010). This is because NH₃ reacts with nitric and sulfuric acids in the atmosphere to form secondary aerosols that, after deposition, might cause eutrophication of N-sensitive ecosystems, toxicity in vegetation, leading to acidification (ERISMAN et al., 2007; TURNER et al., 2010). The N depositions also acts as a secondary source of two greenhouse gases, nitric oxide (NO) and nitrous oxide (N₂O), which contribute to global warming and ozone layer depletion (ERISMAN et al., 2007; SANZ-COBENA et al., 2012).

As other N sources have high cost per N unit and the thick straw layer covering the soil hampers the urea incorporation into the soil, an alternative to reduce volatilization losses of NH₃ when urea is used in sugarcane fields is the amendment of urea by adding the urease inhibitor NBPT (N-(n-butyl) thiofosforic triamide). This compound is an structural analog of urea and competes with the active sites of the enzyme urease, slowing the fertilizer hydrolysis (MANUNZA et al., 1999). The lower rate of hydrolysis avoids sharp pH rises around the fertilizer granule as well as the formation of high concentrations of NH₃ in topsoil. In addition, it ensures a longer time for urea interaction with the soil and occurrence of rainfall to move the urea downwards, thus, reducing the NH₃ loss potential (BOUWMEESTER; VLEK; STUMPE, 1985; WATSON; STEVENS; LAUGHLIN, 1990; GRANT et al., 1996).

Treating urea with NBPT has potential to reduce NH₃ losses when applied over the straw. Under controlled conditions, reduction in NH₃ losses promoted by treating urea with NBPT can be as high as 89% of the maximum loss, and average reductions of 60% are common (SAN FRANCISCO et al., 2011; SOARES; CANTARELLA; MENEGALE, 2012). Under field conditions, similar reduction in NH₃ losses have been observed in crops such as corn and pastures (CHIEN; PROCHNOW; CANTARELLA, 2009). However, in the GCTB system of sugarcane production, the average reduction in NH₃ volatilization average 35% (CANTARELLA et al., 2008).

The low efficiency of NBPT when urea is surface-applied in GCTB systems can be attributed to the high urease activity promoted by the straw layer and the high temperatures of upper soil layers, since urease activity increases as the temperature rises (MOYO; KISSEL; CABRERA, 1989; DHARMAKEERTHI; THENABADU, 1996; TASCA et al., 2011). Moreover, the high temperatures of tropical climate accelerate the NBPT degradation, what can diminish the NBPT efficiency in reduce NH₃ losses (BOUWMEESTER; VLEK; STUMPE,

1985; CARMONA; CHRISTIANSON; BYRNES, 1990; SUTER et al., 2011). This is an indicator that greater amount of inhibitor may be necessary under such conditions.

Studies investigating the effect of increasing NBPT rates on urea reveals that concentrations higher than 240 to 500 mg kg⁻¹ do not reflect in significant increase in inhibition under temperate climates (RAWLUK; GRANT; RACZ, 2001; WATSON et al., 2008). However, Carmona, Christianson and Byrnes et al. (1990) indicate that under high temperatures and plant residues, increasing the NBPT rate can result in increased inhibition efficiency under controlled conditions. Furthermore, the relatively low NBPT efficiency verified in studies carried out in Brazilian sugarcane fields indicate that higher NBPT rates may be required to effectively reduce NH₃ losses under GCTB conditions (CANTARELLA et al., 2008).

Therefore, this study hypothesized that an increase of NBPT concentration in NBPTtreated urea is necessary to reduce volatilization losses and improve the efficiency of this fertilizer in GCTB systems in Brazil. We aimed to evaluate, under field conditions, NH₃ volatilization losses from urea amended with NBPT concentrations and applied over sugarcane straw.

2 LITERATURE REVIEW

2.1 The green cane trash blanketing (GCTB) system

The sugarcane (*Saccharum* spp) cultivated area has increased in Brazil, being currently estimated at about 9 million of hectares (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2015). São Paulo state is the largest producer with 52% (4.68 million hectares) of planted area. Since the beginning of 2014, the burning is no longer allowed in sugarcane farms and sugar mills that have signed the Environmental Protocol with the Government of the State of São Paulo (SÃO PAULO, 2014). From 2016 on, 80% of the mechanized area must be harvested without burning, meeting the provisions of law 11.241 of 19 September 2002. Thus, GCTB is the main system of sugarcane growing in Brazil and all farms will adopt conservative practices in the near future. The amount of straw added annually to sugarcane fields in the GCTB system ranges from 10 to 30 Mg ha⁻¹ of dry material (VITTI et al., 2007), being common 10 to 12 Mg ha⁻¹ after the first harvest.

There is a trend of farmers collect part of the straw for bioenergy production (SORDI; MANECHINI, 2013), but a fraction of the plant material will remain on soil for agronomic reasons. Among the benefits of maintaining the straw on the soil surface are the attenuation of soil erosion by reducing the impact of raindrop, enhancement of water infiltration and reduction of runoff; lower emission of greenhouse gases (compared to burned fields); increase in the soil organic carbon stock and; maintenance of soil moisture along drought seasons (CANTARELLA; ROSSETTO, 2012; CANTARELLA et al., 2013).

On the other hand, the GCTB system can favor some sugarcane pests, such as sugarcane weevil (*Sphenophorus levis*), leafhoppers (*Mahanarva fimbriolata*) and sugarcane borer (*Diatraea sacharallis*) (CANTARELLA et al., 2013). Furthermore, the straw layer hinders certain farming practices, including N fertilizer management. The straw layer can promote N immobilization in the soil due to the high C:N ratio of straw, and enhances NH₃ losses from urea-based N fertilizers susceptible to volatilization when it is broadcast or banded over the straw (COSTA; VITTI; CANTARELLA, 2003; CANTARELLA et al., 2008; CHEN et al., 2008).

2.2 Nitrogen fertilization and NH₃ losses

Nitrogen is an essential element for plants, being component of proteins, amino acids and chlorophyll molecule (MALAVOLTA, 2006). In sugarcane crop, this nutrient is fundamental to ratoons' tillering, productivity and longevity. Given the large N extraction by sugarcane, averaged in 1.2 kg Mg⁻¹ of upper part fresh weight, yield 120 Mg ha⁻¹ of stalks can result in N uptakes as high as 180 kg ha⁻¹; from that amount roughly half is exported through the stalks (OLIVEIRA et al., 2007; OLIVEIRA et al., 2010). Therefore, the usual N rates applied to the ratoon range from 80 to 140 kg ha⁻¹ of N in high yielding systems of Brazil.

Urea is the main N source used in Brazilian agriculture, currently accounting for 60% of the total N fertilizer consumed. This is attributed to high N concentration on this fertilizer, reducing the transport and application costs; and its high solubility, lower corrosivity and compatibility with many other fertilizers if compared to other N sources (GARCIA et al., 2011). However, when applied on the soil surface without incorporation, urea can present N losses by volatilization of NH₃ due the reaction mediated by urease enzyme (MIKKELSEN, 2009; TASCA et al., 2011), which can compromise its effectiveness and, consequently, its economic advantage (LARA CABEZAS; KORNDORFER; MOTTA, 1997; ZAMAN et al., 2008). Besides the economic disadvantage, there is an environmental issue involving NH₃ loss in crop fields, since this gas reacts with nitric and sulfuric acids forming ammonium composts and airborne particles more persistent in atmosphere and easily carried downwind (BOUWMAN et al., 1997; ANDERSON; STRADER; DAVIDSON, 2003). These particles can cause directly damage to the lungs when inhaled and, after deposition contribute to eutrophication and acidification of aquatic and terrestrial ecosystems and to nitrous oxide formation, enhancing the greenhouse effect (ANDERSON; STRADER; DAVIDSON, 2003; ERISMAN et al., 2007; CHEN et al., 2008).

Urease is an enzyme found in plants, bacteria, fungi, invertebrates which exerts catalytic function triggering the urea hydrolysis, resulting in ammonia and carbamic acid that, on account of the spontaneous hydrolysis of the latter, forms ammonia and carbonic acid (KRAJEWSKA, 2009) as shown in eq. (1) and (3). The NH₃ produced neutralizes the active acidity resulting in elevation of pH surrounding the fertilizer granules (eq. (2)), while carbonate buffers the reaction in a pH range from 8 to 9. As the active acidity is neutralized, more NH₃ forms prevail resulting in NH₃ volatilization losses as water is evaporated from soil solution (ROCHETTE et al., 2009).

$$(NH_2)_2CO \xrightarrow{\text{urcase}} H_2N-COOH + NH_3 \xrightarrow{\text{spontaneous}} H_2CO_3 + 2NH_3$$
(1)

$$2NH_3 + 2H_2O \xrightarrow{}_{pKa=9.2} 2NH_4^+ + 2OH^-$$
⁽²⁾

$$CO_2 + H_2O \xrightarrow{} H_2CO_3 \xrightarrow{} H_2CO_3 + H^+ \xrightarrow{} CO_3^- + 2H^+$$
(3)

Ammonia volatilization loss is regulated not only by urease enzyme, but also by soil factors such as soil pH, buffering capacity, cation exchange capacity (CEC) and soil texture. Environmental conditions such as temperature, precipitation, soil water content and wind velocity, as well as size of the fertilizer granules and method of application also affect NH₃ volatilization from urea (BOUWMEESTER; VLEK; STUMPE, 1985; MIKKELSEN, 2009).

Rainfall events or irrigation from 10 to 14 mm right after fertilizing contributes for reducing NH₃ volatilization in bare soil by incorporating the urea into the soil and promoting the vertical and lateral movement of NH₄⁺ formed. This movement reduces the NH₄⁺ concentration near superficial soil layer and moves this ion away from the high pH area surrounding the fertilizer placement site and leads to a greater contact between the NH₄⁺ and the soil CEC in order to retain it in the negative charges (DAWAR et al., 2011a; SANZ-COBENA et al., 2011). On the other hand, Cantarella et al. (2008) report that the required rainfall may be as high as 49 mm to eliminate NH₃ volatilization in GCTB systems. Freney et al. (1994) attribute the higher amounts of water needed to incorporate urea in straw covered soils to the formation of preferential flow channels on straw layer that deviates the water from the urea granules and hinder its incorporate the urea can increase the NH₃ losses by providing moisture for hydrolysis, as reported by Sanz-Cobena et al. (2011) who observed an increase of 8% in ammonia volatilization after irrigating 3 mm after fertilizing.

The soil moisture content is determinant to NH₃ losses. Under dry soil conditions the volatilizations losses are practically null due to the inhibitory effect of the lack of humidity over the urease activity (TASCA et al., 2011). The urease activity increases with increasing soil water content up to near field capacity, followed by a decreasing trend thereafter (DHARMAKEERTHI; THENABADU, 1996). Besides the more water be available to hydrolysis reaction, as the moisture content of a dry soil increases to the field capacity, more quickly urea is hydrolyzed because this helps the urea to diffuse and reach the soil urease (ZHENGPING; VAN CLEEMPUT; BAERT, 1996).

Under wind undisturbed conditions, a high moisture content in soil can even reduce the NH₃ volatilization, since it facilitates the downward transport of NH₄⁺ into the soil (LIU; LI; ALVA, 2007). However, under field conditions where the soil water ascends vertically trough evaporation and is removed by wind, high NH₃ concentration in soil solution leads to NH₃ losses proportional to the water loss rate (TERRY et al., 1978; FRENEY et al., 1992).

In fact, Terry et al. (1978) confirmed that low-relative-humidity air flows over the soil surface enhance the water outflow rate and the NH₃ loss. However, it is only valid when the

urea had already hydrolyzed. A quick drying of the soil surface, enough to inhibit the urease activity before the urea hydrolysis and create a topsoil dry-crust that reduces the rate at which underlying urea moves back to the soil surface can reduced NH₃ losses (BOUWMEESTER; VLEK; STUMPE, 1985). High temperatures also favor NH₃ volatilization since enhance the urease activity, the fertilizer granules dissolution and the evaporation from the soil solution (WATSON et al., 1994b; LARA CABEZAS; KORNDORFER; MOTTA, 1997; RAWLUK; GRANT; RACZ, 2001; CANTARELLA, 2007).

Soil characteristics that affect cation exchange capacity (CEC), such as texture, organic matter content, pH value and buffering capacity can influence in NH₃ losses. In acidic soils with greater ability to supply H^+ ions to the solution in order to neutralize the OH⁻ ions from the urea hydrolysis, N tends to remain in the form of NH₄⁺, reducing the potential of NH₃ loss. Watson et al., (1994b) evaluated the volatilization of ammonia in 16 soils and concluded that in clay soils and with more organic matter content, NH₃ losses tend to be smaller. This can be attributed to the higher buffering capacity, that reduce changes in soil pH surround the urea granule leading to a predomination of NH₄⁺ form, and to the greater capacity of soil CEC in retain the formed NH₄⁺ (CANTARELLA, 2007; SAN FRANCISCO et al., 2011).

Regarding the influence of the size of the urea granules on ammonia volatilization, Werneck et al. (2012), Khalil et al. (2006) e Nömmik (1973) verified lower NH₃ volatilization losses as the urea granule size was increased. Nömmik (1973) attribute that to the retarded hydrolyses by a slower dissolution of the large-pellet urea, enhancing the chance to occur a rainfall before the urea be entirely hydrolyzed, and so burry the fertilizer. Largest NH₃ volatilization are also observed when the fertilizer is banded applied, what can be related to the concentration of fertilizer N in a smaller area, increasing the concentration of NH₄⁺ near a high pH zone (VITTI et al., 2007; ROCHETTE et al., 2009).

Soil management that leave plant material over the soil, as in no-tillage system, sugarcane harvested without burning and perennial crops managed with crop residues, tend to present higher ammonia losses due to the higher urease activity (TASCA et al., 2011; SOARES; CANTARELLA; MENEGALE, 2012). Plants are rich sources of urease which activity remains even after air-drying (FRANKENBERGER; TABATABAI, 1982). Although plant materials may directly provide this enzyme to soil, the increased levels of urease activity in the organic amended soil has generally been attributed to the increased microbial biomass (DHARMAKEERTHI; THENABADU, 1996). According to Zantua e Bremner (1976), any organic material that promotes microbial activity will increase urease activity in soils if added in sufficient quantity, and this increase will be proportional to the amount of material added.

In addition to promoting ureolytic activity, the layer of straw on the soil surface also favors the NH₃ loss acting as a barrier between fertilizer and soil, hindering the diffusion of urea into the soil, what makes the NH₃ volatilization a major problem in the sugarcane harvested without burning urea is used (VITTI et al., 2007). Several studies have been performed under such conditions reporting NH₃ losses ranging from 1 to 11% of the applied N under rainy conditions (CANTARELLA et al., 2008) and from 15 to 36% under favorable conditions of NH₃ volatilization (COSTA; VITTI; CANTARELLA, 2003; VITTI et al., 2007; CANTARELLA et al., 2008).

The fertilizer incorporation into the soil reduces substantially NH₃ volatilization losses (ROCHETTE et al., 2009; TASCA et al., 2011). However, in the GCTB system, the large amount of straw on the soil surface makes the incorporation a difficult, expensive (CANTARELLA et al., 2008), and frequently inefficient practice.

The use of nitric or ammoniacal N-sources, which risk of volatilization in acid soils (majority of Brazil's soils) is virtually null, is an alternative to overcome the loss of NH₃. However, the high cost of these sources and legal restrictions in transport of ammonium nitrate because of its explosive potential, have increased interest in the use of urea treated with volatilization inhibitors that come to increase the efficiency of the surface applied N fertilizers (CANTARELLA et al., 2008).

2.3 Urease inhibitor NBPT

Urease inhibitors are an alternative to improve the efficiency of surface-applied urea under GCTB systems (CANTARELLA et al., 2008; BARTH, 2009; SOARES; CANTARELLA; MENEGALE, 2012). Urease inhibitor is a compound that, when added to fertilizer, retard urea hydrolysis. This increases the time available to a rainfall occur and incorporate urea, reducing the NH_3^+ formation near soil surface and hence reducing the NH_3 loss potential (GRANT et al., 1996; WATSON et al., 2008).

Many products, including metals, boron and organic compounds have been evaluated to inhibit urease activity (CHIEN; PROCHNOW; CANTARELLA, 2009; KRAJEWSKA, 2009; UPADHYAY, 2012). However, according to Watson et al. (2008) a few products comply the key characteristics required, such as efficiency under low concentration, non-toxicity, stability, compatibility with urea and low cost. Among the currently available inhibitors, N-(n-butyl) thiophosphoric triamide (NBPT) is the most effective and one of the most studied, being commercialized in the USA since 1996 (KRAJEWSKA, 2009; CANTARELA et al., 2008; WATSON et al., 2008).

The NBPT is not a direct urease inhibitor. Tests of the pure NBPT in vitro have shown its total ineffectiveness (CREASON et al., 1990). After contact with soil or plant material, NBPT is converted to its oxygen phosphate analog N-(n-butyl) phosphoric triamide (NBPTO), which is the actual inhibitor. However, NBPTO is not stable enough to be used to treat urea directly or to be packaged and distributed for commercial use, whereas NBPT is quite stable (HENDRICKSON; DOUGLASS, 1993).

Consequently, the efficiency of NBPT in soil is directly associated with the aeration and moisture content of soil (BYRNES; FRENEY, 1995; CHIEN; PROCHNOW; CANTARELLA, 2009; JUAN et al., 2009). In aerated soils, there is a rapid conversion of NBPT to NBPTO. NBPTO have a structure similar to urea allowing inhibition of urease activity by means of competing with the specific substrate for the active sites of this enzyme, binding strongly to three active sites of urease molecule (MANUNZA et al., 1999; WATSON et al., 2008; CHIEN; PROCHNOW; CANTARELLA, 2009). This three-point binding is the key of NBPT efficiency as inhibitor.

By slowing urea hydrolysis, NBPT avoids sharp pH raises surrounding the urea granule, avoiding NH₃ formation, and allowing more time for a rainfall occurs to dissolve and move the urea molecule below the soil surface (GRANT, 2014). Once urea is hydrolyzed deeply in soil, the NH₃ formed is protected from volatilization by interaction with soil acidity, forming NH₄⁺ which is retained in soil CEC, and by the nitrification process that quickly takes place of ammonification (CHRISTIANSON et al., 1993; ZHENGPING; VAN CLEEMPUT; BAERT, 1996; DAWAR et al., 2011a). Since NBPT delays rather than eliminates urea hydrolysis, its effectiveness in reducing volatilization will depend on many factors that intensify the hydrolyze and diffusion of urea in the soil (CHRISTIANSON et al., 1993).

NBPT can inhibit urea hydrolysis for periods from 7 to 14 days depending on soil attributes such as pH, initial moisture, temperature, and other environmental conditions (HENDRICKSON; DOUGLASS, 1993; SANZ-COBENA et al., 2008; DAWAR et al., 2011a). Hendrickson and Douglass (1993) observed a complete inhibition of urea hydrolysis up to 14 days after fertilizer application, being this period directly related to the concentration of inhibitor blended into urea. However, in most cases the NBPT treatment delays the time of the maximum rate of NH₃ loss to the seventh or ninth days, while for urea untreated this peak occur around third and fourth day (WATSON et al., 1994b; SANZ-COBENA et al., 2008; DAWAR et al., 2011a; SOARES; CANTARELLA; MENEGALE, 2012). The inhibition period of NBPT applied under Brazilian conditions is still not clear, specifically following application over large amount of crop residues such as in GCTB systems.

Treating urea with NBPT reduces, but does not completely control NH₃ losses when urea is surface-applied. The reduction of NH₃ losses of urea-treated NBPT ranges from 45% to 85% of the maximum loss that occur for untreated urea (WATSON et al., 1994b; SANZ-COBENA et al., 2008; CHIEN; PROCHNOW; CANTARELLA, 2009; SOARES; CANTARELLA; MENEGALE, 2012). The NBPT efficiency is variable depending on factors such as product storage conditions, environmental conditions and soil physicochemical characteristics.

Regarding the degradation during storage, accordingly the manufacturer, the stabilized fertilizer may be stored up to 9 months for full effectiveness under temperatures between -18 °C and 38 °C (KOCH AGRONOMIC SERVICES, 2015). However, Watson et al. (2008) reported that the half-life of NBPT was only 74 days when it was coating urea.

Once applied in field, the NBPT stability is dependent on soil temperature and soil pH. Considerable loss of NBPT efficiency was observed when it was used under high temperatures and acidic soils (HENDRICKSON; DOUGLASS, 1993; ENGEL et al., 2013). That indicates that decomposition of the inhibitor in acidic soils is generally complete before the soil pH increased sufficiently to slow further decomposition. As a result of increased soil temperature, NBPT degrades quickly (ENGEL et al., 2013) whilst urease activity in soils increase, demanding more inhibitor to occupy the great number of active sites (CARMONA; CHRISTIANSON; BYRNES, 1990).

Furthermore, other authors reports lower efficiencies of NBPT-treated urea under high urease activity (CHIEN; PROCHNOW; CANTARELLA, 2009; TASCA et al., 2011), waterlogged conditions (ZHENGPING et al., 1991) and low clay content in soil (RAWLUK; GRANT; RACZ, 2001; SAN FRANCISCO et al., 2011). Under high temperatures and initial moisture conditions, the NH₃ ascends with the water flow to the soil surface and is lost to the atmosphere (SANGOI et al., 2003; DA ROS; AITA; GIACOMINI, 2005; SAN FRANCISCO et al., 2011).

In conditions of low rainfall, like in the dry season in tropical environments, the efficiency of the urease inhibitor tends to be lower. In the dry season of Brazil, Cantarella et al. (2008) observed a reduction of 25% in NH₃ losses from NBPT-treated urea as compared to urea, while during the rainy season the reduction was 57% when fertilizers were applied over sugarcane straw. According to the authors, the results suggest that under dry season the NBPT had been degraded before the fertilizer was incorporated into soil, thus subjecting urea to NH₃ volatilization losses.

The NBPT is currently commercialized in Brazil under the brand "Agrotain®"

(KochTM). The formulation of Agrotain[®] contains 30% of NBPT and allows mixing with Nbased fertilizers already produced (KOCH AGRONOMIC SERVICES, 2015), being normally used for coating urea granules. This commercial formulation has been used in the most studies performed in Brazil under proportion NBPT/urea from 530 to 1000 mg kg⁻¹ or 0,053 to 0,1% w/w. The most widely commercial product in Brazil (Super N[®]) contains a NBPT concentration of 530 mg kg⁻¹.

Evaluating NBPT concentrations (0, 500, 1000 and 1500 mg kg⁻¹) on surface applied urea (100 kg ha⁻¹) under Canadian conditions, Rawluk, Grant and Racz (2001) observed that, in most of cases, increasing NBPT rates beyond 500 mg kg⁻¹ did not result in a large reduction of NH₃ losses. Nevertheless, Carmona, Christianson and Byrnes (1990) observed that, under warm temperatures, the effect of NBPT in reducing cumulative NH₃ losses was only obtained when higher doses were used. In both studies, authors have pointed that, under high temperature and high urease activity conditions, the urea hydrolysis rate might overcome the NBPT conversion rate to NBPTO, or the inhibitor degradation rate can be quicker and hence higher NBPT concentration may be necessary for an adequate level of inhibition. This is in agreement with evidences that while NBPT-treated urea reduced NH₃ volatilization losses from 60 to 70% in crops such as maize and pasture during rainy spring and summer periods (CHIEN; PROCHNOW; CANTARELLA, 2009), the reduction in losses averaged 35% in GCTB systems in Brazil (CANTARELLA et al., 2008).

In this systems, the large amount of straw left over soil surface supports a great activity of the enzyme urease and can promote degradation of the inhibitor (SOARES; CANTARELLA; MENEGALE, 2012). Moreover, in the state of São Paulo, the largest Brazilian producing region, sugarcane ratoon fertilizing is usually carried out from May to December, right after the harvest. It coincides with the dry season, when rainfall is limited, decreasing the chances of rapid urea incorporation by rain (CANTARELLA et al., 2008). The result is that, apart from the advantages of using urea, most sugarcane growers prefer to use N sources less subject to NH₃ volatilization losses, such as ammonium nitrate. Therefore, an increase of NBPT concentration in urea above 530 mg kg⁻¹ may be required to effectively reduce NH₃ losses and makes viable the usage of NBPT-treated urea in GCTB sugarcane fields in Brazil.

3 MATERIAL AND METHODS

3.1 Experimental sites

Six field trials were carried out across the State of São Paulo, between late May, 2014 and early November, 2015. Experiments were implanted in commercial areas of sugarcane ratoon harvested without burning (GCTB), in order to obtain representative results under diverse soil and climate conditions along the sugarcane harvest period (Table 1).

Soil samples were collected to a depth of 20 cm before fertilizer application in order to determine physical and chemical soil properties (Table 2). Chemical analysis were performed according to Raij et al. (2001) and physical analysis according to Camargo et al. (2009). Biomass of straw was also measured before fertilizer application, by means of collecting the straw present in 1 m² in 10 random positions in the area. The straw was weighed in the field to determine fresh weight and subsample was dried under 75 °C for 72 hours to determine moisture and used to estimate straw dry mass. The straw attributes are listed in Table 3.

Each experiment lasted for 30 days. During this period, data of temperature, relative humidity and rainfall were collected in all areas (Table 4). Rainfall data was collected using a digital pluviometer set up in the experimental area. The remaining weather information were collected from nearest automatic meteorological stations.

Field trial	Local	Coordinates	Month	Weather condition	Soil
1	Irocománolia	22°35'18.0"S	$J_{\rm upo}/2014$	Dur	Clay
1	fracemapons	47°31'30.2"W	Julie/2014	Dry	Clay
2	Directorho	22°38'07.2"S	$O_{at}/2014$	Daimy	Sandy
	Piracicada	47°45'49.7"W	000/2014	Kalify	Sandy
3	Aronos	22°24'38.9"S	$D_{22}/2014$	Dainy	Clay
	Alalas	47°24'38.9"W	Dec/2014	Kalliy	Cidy
4	Direciache II	22°47'22.8"S	$M_{ox}/2015$	Dmr	Loom
4	Piracicada II	47°35'48.0"W	Way/2015	Dry	Loam
5	Datinandaha	21°2'56.8"S	$\Delta u_{2}/2015$	Darr	Laam
3	Potirendada	49°30'48.6"W	Aug/2015	Dry	Loam
6	Itinonino	22°11'41.4"S	Samt/2015	Derry	Sandy
6	nirapina	47°47'12.5"W	Sept/2015	Dry	Sandy

Table 1 - Location of field trials in São Paulo, 2014-2015

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Field trial	pН	Р	S	K	Ca	Mg	Al	H+Al	SB	CEC	V	m	Sand	Silt	Clay
		mg e	dm ⁻³				mmol	c dm ⁻³ —			%	6		g kg ⁻¹	
1	5.5	40	7	3.2	44	19	0	38	66.2	104.0	64	0	161	130	708
2	4.1	2	12	1.4	6	3	6	38	10.4	48.4	21	37	884	16	100
3	4.9	14	32	2.3	27	11	2	42	40.3	82.3	49	5	200	131	669
4	5.0	11	5	0.4	29	11	0	22	40.0	62.4	65	0	748	27	225
5	4.9	5	6	0.6	8	4	2	20	12.6	32.6	39	14	752	21	227
6	5.5	43	8	0.6	34	8	0	13	42.6	55.6	77	2	887	13	100

Table 2 – Soil chemical and physical attributes at 0 - 20 cm depth in each field trial

Table 3 – Fresh weight and dry matter of sugarcane straw in each field trial before fertilizer application

Field Asial	Straw dry mass	Straw moisture			
riela triai	(kg ha ⁻¹)	(%)			
1	13,800	11.8			
2	10,860	3.6			
3	15,840	20.0			
4	9,756	27.8			
5	14,670	16.3			
6	17,252	37.2			

 Table 4 – Average values of temperature (T), relative humidity (RH) and experimental days with rain and accumulated rainfall during the 30 days of experiment evaluation

Field trial												
	1	2			3		4		5	6		
Т (°С)	RH (%)	Т (°С)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	
18.5	85	25.4	74	23.1	84	20.6	94.3	22.2	17	24.4	55.9	
Day	Rain (mm)	Day	Rain (mm)	Day	Rain (mm)	Day	Rain (mm)	Day	Rain (mm)	Day	Rain (mm)	
11	3.9	12	2.6	3	3.0	2	0.4	25	0.5	9	15.7	
12	5.7	17	4.3	13	4.0	8	0.5	-	-	11	8.8	
13	1.2	18	7.3	15	36.0	12	15.7	-	-	23	8.1	
17	1	19	15.0	16	12.0	14	31.7	-	-	25	0.7	
24	9.2	25	2.3	26	3.0	-	-	-	-	-	-	
25	2.3	26	32.1	29	40.0	-	-	-	-	-	-	
30	0.8	28	21.2	-	-	-	-	-	-	-	-	
Total	24.1		84.8		98		48.3		0.5		33.3	

3.2 Experimental design

The experiments were set up in a randomized block experimental design with seven treatments and four replications. The treatments consisted of urea (45% of N) amended with NBPT rates of 0, 530, 850, 1500 and 2000 mg kg⁻¹, ammonium nitrate (32% of N) and a control plot (without N), as shown in Table 5.

Treatment	Description
1	Control
2	Urea
3	Ammonium nitrate
4	Urea amended with NBPT (530 mg kg ⁻¹ of NBPT)
5	Urea amended with NBPT (850 mg kg ⁻¹ of NBPT)
6	Urea amended with NBPT (1500 mg kg ⁻¹ of NBPT)
7	Urea amended with NBPT (2000 mg kg ⁻¹ of NBPT)

Table 5 – Description of treatments

3.3 Measurement of NH₃ volatilization

It was adopted the enclosure method of volatilization chambers similar to those described in Nömmik (1973) and Cantarella et al. (2008). The chambers consisted in PVC hollow cylinders, 20 cm in diameter and 40 cm in height, containing two polyethylene foam disks, treated with a H_3PO_4 + Glycerol solution in order to convert into NH_4^+ and trap the NH_3 released from the soil surface. The upper end of the cylinder was covered with a plastic cap that allowed air passage but protected the discs from the rain. The PVC chambers were fitted on top of PVC bases, which were cylinders 19 cm in diameter and 20 cm in height that were partially inserted into the soil so that around 10 to 15 cm of their length were left above ground.

Foams were previously cleaned by means of sequentially washing in a KOH solution $(0.5 \text{ mol } L^{-1})$, H₃PO₄ solution $(1 \text{ mol } L^{-1})$ and deionized water. After drying, each foam disk was soaked with about 80 ml of a solution 0.75 mol L⁻¹ H₃PO₄ and Glycerol (4% v/v). After treatment, foams were packed in pairs in plastic bags and frozen. Two hours before use, the foam discs were unfrozen. The first disc was arranged about 15 cm from the ground, hermetically sealing the collecting chamber formed by perfect fit between the collector and the fixed base. The second disc was allocated just above, about 2 cm. The upper foam was installed to avoid cross contamination with NH₃ from atmosphere or from other treatments.

In each plot, eight bases were installed at 20 cm from the sugarcane row and 20 cm from the next base, performing a semi-static collecting system (static bases with a collector interchangeable between bases). Since the chambers do not allow the rain reach the fertilizer placed within, at each foam replacement, the chambers were moved to the next base, returning to the initial and second base to perform the ninth and the tenth collections, respectively. Hence, the NH₃ loss assessment of the following period were made from a fertilizer treatment exposed to the same conditions (rain, temperature, wind, humidity etc.) as the rest of the field.

3.4 Fertilizer treatment and application

The treatment of urea with NBPT was performed using the same amount of solution with different NBPT concentrations, in order to reduce the vehicle influence. Thus, for each kilogram of urea were added 4.00 g of NBPT solution containing the respective concentrations to reach the target concentration of each treatment. The mixture and homogenization was performed by means of manual shaking the mixture of urea plus NBPT solution inside polyethylene bags during 1 min. The NBPT solutions were provided by KOCHTM.

The fertilizers were individually weighed and applied over the straw inside the PVC bases previously installed in the area. The straw around each base was cut using sharp knifes to allow its partial insertion into soil and preserving the straw cover characteristic of area.

Within each base assigned to receive N treatments, the respective fertilizer were added in an amount corresponding to 3000 mg of N. Considering the diameter 0.2 m of the chambers, it is equivalent to a fertilizing with 100 kg ha⁻¹ of N banded applied (0.10m) in a sugarcane field 1.5 m spaced. Every base received 6.00g of potassium chloride, equivalent to 120 kg ha⁻¹ of K₂O, simulating usual ratoon fertilization practices.

3.5 Foams collecting, extraction and N analysis

Sampling and replacement of the foam discs were carried out at 2, 4, 6, 8, 10, 12, 16, 20, 25 and 30 days after fertilization (DAF). Once collected, the foams were packed in plastic bags, tagged and frozen until the extraction time. To extract the NH₃ trapped into the foams, each disk was put into a 2 L beaker plus 400 mL of deionized water. The foam were soaked and drained several times aiming to homogenize the N concentration in solution. The solution was manually extracted from the foam and both beaker with solution and drained foam were weighed in a precision scale (error = 0.01 g). The mass of the beaker and one dry foam was discounted to obtain the amount of solution retained in each single foam.

An aliquot of the solution remaining in beaker was collected and frozen until analysis (approximately 80 mL). After defrosting, N concentration was determined colorimetrically by Flow Injection Analysis (FIA), at the Ismatec[®] Automated Sample Injection Analyzer (ASIA), according to Kamogawa and Teixeira (2009). The principle of the method was based on the contact of the sample containing NH₄⁺ ions with a solution of NaOH 1 mol L⁻¹, forming NH₃ which diffuses through a polypropylene membrane selective to gas into a diffusion chamber where NH₃ reacts with an indicator solution of bromocresol purple (pH \approx 6.5). In contact with NH₃, the solution pH raises and the indicator color changes from wine-colored to purple. The solution flows into a quartz cuvette where the color intensity is monitored by a photometric

detector at a wavelength of 605 nm. The N concentration is obtained by correlating the area under the transient peaks and the concentration of ammonium sulfate reference solutions. The amount of volatilized N-NH₃ from each collect was obtained through eq. (4):

$$N_{(mg)} = C_{(mgL^{1})} \times \frac{M_{(g)}}{d_{(gL^{1})}}$$

$$\tag{4}$$

where *N* is the amount of N-NH₃ volatilized; *C* is the concentration of N in the aliquot; *M* is the mass of the extracted solution obtained by the weight difference between (mass of foam + beaker + solution) - (dry and empty beaker + dry foam weight) and; *d* is the density of the extracted solution (it was considered 1000 g L^{-1}).

Ammonia loss was expressed as percentage of the N applied in each single period per treatment. In order to verify the NH₃ volatilized from fertilizers, the average volatilization of control plots was subtracted from all fertilized treatments.

3.6 Statistical analysis

For each local, cumulative loss of NH₃ at the end of 30 DAF submitted to ANOVA by F test (P<0.05). The residuals variances of the locals were compare using Hartley test (P<0.05) and, with the locals with homogeneous residual variance, were carried out a combined analysis (P<0.05) of experiments in randomized nested blocks, with locals and NBPT concentration as factors. The means between locals were compared by Tukey HSD test (P<0.05) and the effect of NBPT concentrations were tested by regression analyses (P<0.05). The percentage of inhibition of NH₃ loss by NBPT was calculated by ((U - I) $\div U$) ×100, where U is the NH₃ loss from urea and I is the NH₃ loss from urea amended with the inhibitor.

To study the treatments volatilization trends along the time, a Boltzmann sigmoidal model modified from Navarro-Verdugo et al. (2011) were used (eq. (5)). This sigmoidal model has an increasing monotone shape and, among other sigmoidal models has the potential to fit the cumulative volatilization pattern where, after an initial moment of low or even none NH_3 loss, the urease activity triggers a lag phase of intense volatilization that, after a transition point, loose intensity as the substrate is exhausted and nitrification reaction prevail. Furthermore, the modified Boltzmann sigmoidal model is symmetric and its parameters have a directly practical interpretation as shown in eq. (5):

$$\hat{y} = \frac{a}{\left(\frac{t_0 - t}{b}\right)}$$
(5)

where \hat{y} is the cumulative NH₃ loss in percentage of applied N, *t* is the time after fertilizing in days and *a*, *b* and *t*₀ are the parameters of the model. This parameter has the following interpretation: *a* is the maximum NH₃ loss accumulated in the period; *t*₀ is a three interpretation parameter being the time when 50% of the maximum loss occurs, the moment when the maximum rate of loss occurs, and the inflexion point of the function; *b* describes the behavior of the slope of the process during the transition. Despite the lack of direct interpretation, the parameter *b* can be used to estimate the maximum rate of loss (R_{max}) at the *t*₀ moment through the eq. (6):

$$R_{\rm max} = \frac{a}{4b} \tag{6}$$

Curves were fitted to cumulative daily NH₃ volatilization for each of the four replicates per treatment for each local. A combined analysis was carried out for all locals with homoscedastic residual variance and paired comparisons were performed to determine differences for a parameter means between treatments inside each local with a greater data set in order to enhance the power of ANOVA. To perform the comparisons was used the Tukey HSD Test (P<0.05).

The sigmoidal curves were fit and the parameters estimated using the dynamic curve fit function of SigmaPlot 12.5 (SYSTAT SOFTWARE, 2013) and the command *nls* of R software (R CORE TEAM, 2015). All the other analyzes were performed using the R software.

4 RESULTS AND DISCUSSION

4.1 NH₃ volatilization over time

Results shown in Figure 1 indicate the influence of rainfall on NH₃ volatilization. In Iracemápolis there was no significant losses of NH₃ for any treatment along the first 12 DAF (Figure 1a). In those circumstances, the volatilization only began after 10.8 mm of rain accumulated in three events at 11, 12 and 13 DAF. A similar pattern was found in Araras (Figure 1c) and in the Piracicaba II (Figure 1d), were light rainfalls of 3 mm and 0.5 mm at the second and third DAF, respectively, triggered the losses in untreated urea plots. In Araras, NH₃ volatilization was raised from 0.4% on the second DAF, to 5.6% and 9.8% of the applied N on 4 and 6 DAF, respectively. In Piracicaba II, this variation was from 1.1%, on the second DAF, to 5.9% and 18.4% of applied N on the 4 and 6 DAF, respectively, for untreated urea.

No model was fitted to ammonium nitrate treatment in all trials, or to the whole Potirendaba trial, because the difference of NH₃ volatilized over time and the cumulative loss was virtually null (P>0.05). Furthermore, data of NH₃ loss of Potirendaba was not included in combined analysis because the extremely low volatilization (<0.5% of N-applied) resulted in relatively low variance if compared to the other locals hence not meeting the homoscedasticity requisite. For all other locals, the Boltzman sigmoidal model was highly significant (Annex C).

In the first trial in Piracicaba (Figure 1b), however, NH₃ losses from urea began shortly after application even without rain events, with a peak of losses occurring on the sixth DAF. In this specific case, it can be attributed to rain occurrence and constant rainy and cloudy weather in the region a few days before trial installation. The fertilizers were applied a few days after rainfall events, when the straw was almost dry but the soil was still moisten. That humidity could be enough to start the hydrolysis process and transport the formed NH₃ to atmosphere gradually through the first week. After that, no additional losses were verified until 2.6 mm of rain on 12 DAF have triggered the volatilization for all treatments. In Itirapina (Figure 1f) slight NH₃ emissions occurred in the first six DAF, reaching 3.3% of the applied N, but the main losses for al treatments only started after two rain events on 9 and 11 DAF.

Results from literature indicated that NH₃ losses starts between two and four DAF under controlled conditions with soil previously moisten (WATSON et al., 1994a; SOARES; CANTARELLA; MENEGALE, 2012). However, under low initial soil moisture content, the rainfall is required to provide water to the hydrolysis reaction occurs, as demonstrated herein. Obcemea et al. (1993) observed, under field conditions, extremely low volatilization losses from urea applied over sugarcane trash along 14 DAF, when 3 mm triggered major losses.



Figure 1 – Cumulative NH₃ losses after application of ammonium nitrate and urea amended with increasing rates of NBPT in six field trials. Curves were fitted for each treatment using Boltzmann sigmoidal model. A means a rainy event occurrence

The role of rainfall on providing water to hydrolysis reactions is clear when analyzing data from Potirendaba experiment (Figure 1e). Before fertilizers application, there was a long drought period and no considerable rain were verified along the experimental time. This led to extremely low NH₃ losses in Potirendaba, with a maximum accumulated loss of 1.5% of applied N from urea. Despite the fertilizer granules had dissolved, probably by dew formation, neither

the dew, nor a 0.5 mm rain occurring on 25 DAF, were sufficient to start the volatilization process. The result is intriguing considering that urea application over a large amount of straw resulted in very limited NH₃ losses in a period of 30 DAF.

The first hypothesis is that urea did not undergo hydrolysis under such dry conditions. It is possible that the lack of moisture have impeded the hydrolysis process and thereafter avoided the NH₃ formation and the increase in pH near the fertilizer granules. Such finding is in accordance to the observations of Obcemea et al. (1993), which banding 80 and 160 kg ha⁻¹ of N as urea on sugarcane trash verified little change in the pH or in the ammoniacal N concentration in the straw until a rain event took place.

Furthermore, in our study, any moisture even available through the nightly dew could had been quickly lost as the day went warm and the relative humidity felt in the early morning, stopping the hydrolysis process. In fact, Bouwmeester, Vlek and Stumpe (1985) observed reduction in NH₃ losses from 19 to 7.5% of applied N when urea was applied to a soil at 21% of permanent wilting point that was subjected to rapid soil surface drying thereafter, whereas Engel, Jones and Wallander (2011) reported higher losses of NH₃ when urea application to high-water-content soil surfaces was followed by a period of slow drying.

Given that in our study the largest rates of volatilization occurred during periods of drought or low precipitation after a light rain event have triggered the main losses, another possible explanation is that, even the hydrolysis process have occurred at some extent, NH₃ volatilization was low due to absence of water evaporation from soil solution or from straw, since the NH₃ emissions are directly correlated with water evaporation (FRENEY et al., 1992). Furthermore, the hydrolysis could have occurred in a rate similar to the nitrification, leading to a conversion of ammonium in nitrate before the NH₃ was lost from soil.

The rainfall were also decisive in NH₃ emissions length. While successive lowintensity rainfalls enhanced the volatilization, intense rainfall played an important role in mitigating NH₃ losses. In Piracicaba, Araras and Piracicaba II, the volatilization of urea treatment stopped and the NBPT had its losses reduced only after rainfall events of 32.1, 36.0 and 31.7 mm, respectively. In Iracemápolis, in a clay soil, 11.5 mm accumulated in two rain events at 24 and 25 DAF reduced but not stopped the losses, while in Itirapina, a sandy soil covered with a thick straw layer, 15.7 mm on the 9 DAF only intensified the volatilization process. In bare soil drastically reductions or even zero NH₃ volatilization have been reported after rainfall events or irrigation from 10 to 14 mm (SANZ-COBENA et al., 2011) or >18 mm (ENGEL; JONES; WALLANDER, 2011) occurring right after fertilizing. However, when urea is applied over sugarcane straw, a greater amount of rain may be required, as reported by Cantarella et al. (2008) and Prasertsak et al. (2002) that observed that NH₃ emissions stopped only after 49 mm and 50 mm rainfall events, respectively. According to Freney et al. (1994) in presence of sugarcane straw, the water is shed from straw along preferred pathways, and much of the urea can be protected from dissolution by the rain-water, demanding more intensive rainfall to incorporate the fertilizer into the soil and hence diminish the NH₃ output.

4.2 Effect of NBPT rates in reducing NH₃ volatilization

In Figure 1 it is possible to observe a diminishment in NH_3 volatilization losses as the NBPT rates increases in most sites. Besides volatilization process had been triggered by rainfall for all treatments, NH_3 emissions from urea started as soon there was available moisture in soil, while the onset of NH_3 losses from NBPT-treated urea occurred about four days after that. As a result, a clear effect of NBPT rates on delaying the time of maximum daily loss rate (T_{max}) was verified (Table 6).

Table 6 – Accumulated NH₃ loss (A_{max}), day when occurred the maximum rate of loss (T_{max}), maximum daily rate of loss (R_{max}) of urea amended with NBPT rates in six field trials along 30 days. Parameters of the regression were obtained from a Boltzmann Sigmoid model adjusted for the cumulative losses of NH₃¹

	NBPT rates (mg kg ⁻¹)										
Parameter	0		530)	850		1500		2000		
			— Iracemápo	olis —							
A _{max} (% of applied N)	13.7	a	10.9 b	,	7.5	c	7.4	c	7.2	c	
T _{max} (days)	19.4	c	21.6 b	•	20.9	bc	25.2	a	21.1	b	
R _{max} (% of applied N day ⁻¹)	1.4	a	1.3 a	b	0.7	ab	0.5	b	0.7	ab	
			— Piracicab	oa —							
A _{max} (% of applied N)	32.7	а	28.0 b)	24.7	bc	28.0	b	23.2	c	
T _{max} (days)	12.4	b	16.5 a		16.4	a	17.2	a	16.9	a	
R _{max} (% of applied N day ⁻¹)	2.9	b	5.8 a		4.4	a	5.8	a	3.9	ab	
			—— Araras								
A _{max} (% of applied N)	19.3	a	11.6 b	•	11.0	bc	8.4	c	7.5	c	
T _{max} (days)	7.2	c	10.9 b)	12.1	b	14.1	a	13.9	a	
R _{max} (% of applied N day ⁻¹)	1.8	a	1.2 a	b	1.2	bc	0.9	b	0.8	b	
			— Piracicaba	ι II —							
A _{max} (% of applied N)	29.7	a	17.5 b	•	11.5	bc	10.8	c	9.5	c	
T _{max} (days)	6.3	c	12.0 b	•	12.4	b	13.1	ab	14.2	a	
R _{max} (% of applied N day ⁻¹)	3.8	a	2.1 a	b	1.3	bc	1.0	bc	0.7	c	
			—— Itirapin	a ——							
A _{max} (% of applied N)	19.9	a	17.7 b	•	15.2	bc	13.3	c	11.2	c	
T _{max} (days)	11.8	c	15.4 b	,	15.2	b	18.1	а	18.4	a	
R _{max} (% of applied N day ⁻¹)	3.7	a	2.2 a	b	2.5	ab	1.2	bc	0.9	c	

¹Means not sharing a letter in the same line are significantly different by Tukey's HSD test (P < 0.05)

The T_{max} of urea were variable between locals, ranging from 6 to 19 DAF, and were directly related to rainfall events after fertilizer application as demonstrated in Figure 1. For all locations, the increase in NBPT concentration delayed T_{max} . The lower NBPT concentrations promoted an average delay of 4 days in T_{max} as compared to untreated urea, while for the two higher concentrations the average delay was 6 days. This is slightly greater than observed by Soares, Cantarella and Menegale (2012) and Watson et al. (2008), which obtained around 5 days of delay using urea amended with ~1000 mg kg⁻¹ of NBPT under controlled conditions. It is noteworthy that in such studies the T_{max} occurred between 2 and 4 DAF for urea, once there was adequate moisture conditions to hydrolysis and urea diffusion right at the moment of fertilizer application. In this study even with the onset of NH₃ losses from urea occurring later, six days of delay were still obtained when NBPT was used. This shows that the NBPT efficiency in delaying the T_{max} were not compromised after a longer time of fertilizer exposition to the average field elements.

Under field conditions, delaying T_{max} increases the chance of rainfall occur and hence incorporate the urea into the soil, resulting in lower NH₃ volatilization (WATSON; STEVENS; LAUGHLIN, 1990). Furthermore, even in absence of high rainfall events to incorporate the fertilizer, a wider delay in T_{max} allows a great diffusion of urea away from the fertilizer microsite, if there is enough moisture. This larger diffusion leads to a better buffering of hydrolysis reaction by soil acidity and hence diminish the pH increasing around the fertilizer granule (DAWAR et al., 2011a). This leads to lower formation of NH₃ close to the soil surface, resulting in lower rates of NH₃ loss (CHRISTIANSON et al., 1993).

In fact, for all trials, except Piracicaba, the increase in NBPT concentration reduced the maximum rate of loss (R_{max}). In addition, by favoring urea diffusion, the higher NBPT concentrations may have increased the proportion of urease active sites blocked by the inhibitor when the conditions became favorable to hydrolysis. The lower hydrolysis promoted by the residual NBPT still bounded to enzyme, results in NH₄⁺ formation at a rate close to nitrification rate (ZHENGPING; VAN CLEEMPUT; BAERT, 1996) what reduces the formation of NH₃ and hence diminish the rate of loss.

In Piracicaba, however, the model did not capture the two peaks of losses from urea treatment, especially the second peak, which was sharp. During the first week after fertilizing there was enough soil moisture for the hydrolysis of untreated urea leading to a first peak of volatilization for this treatment, which suddenly stopped after soil drying. Besides, the high urease activity, associated with a high temperatures occurred in the first week after fertilizing, may have promoted a rapid and substantial degradation of NBPT (CARMONA; CHRISTIANSON; BYRNES, 1990; ENGEL et al., 2013). Then, when a light rainfall triggered the volatilization process for all treatments at 12 DAF, the NBPT shortage to promote an adequate inhibition in presence of non-hydrolyzed urea led to high rate of loss at the T_{max} for NBPT-treated urea, if considered the model. However, it is clear the occurrence of two peaks for untreated urea, as sharper as the occurred for NBPT-treated urea.

As a result of the combination of delaying T_{max} and lowering R_{max} , NBPT treatments promoted a substantial reduction of maximum cumulative loss estimated (A_{max}). As there was no significant concentration x local interaction for A_{max} (Annex D), the same pattern of reduction was verified for all locals. It was clear that, even the lower concentration of NBPT was effective in mitigating NH₃ losses if compared with untreated urea.

The combined results among sites indicate an overall reduction in NH₃ volatilization, as percentage of the maximum loss, of 26.0, 38.6, 42.6 and 49.2% for the 530, 850, 1500 and 2000 mg kg⁻¹ NBPT concentrations, respectively. The inhibitory effect of the two higher NBPT concentrations was clearly superior to the lower concentration. That was also slightly superior to the inhibition reported by Cantarella et al. (2008), whose using urea amended with 530 mg kg⁻¹ of NBPT surface applied on sugarcane trash blankets in several trials through rainy and dry seasons, obtained an average reduction of 35% from maximum loss. Hence, despite the mean comparison do not provide a precise definition of the ideal NBPT concentration there is a strong evidence that higher inhibition efficiencies can be reached with the increase of NBPT concentration above the currently concentration of 530 mg kg⁻¹.

The relative inhibition presented great variability between sites. For example, the inhibition promoted by the higher NBPT concentration, was 27, 69, 43, 61 and 49% for Piracicaba, Piracicaba II, Itirapina, Araras and Iracemápolis, respectively. The variable NBPT efficiency in different conditions is apparently related to the intensity of rain events in the days following the onset of losses. When intense rain events (>30 mm) occurred at latest 10 days after loss started, such as in Araras and Piracicaba II trials, NBPT efficiencies at around 65% were observed, whereas when only light rainfall occurred in this meantime, such as in Piracicaba, Itirapina and Iracemápolis, reduction in losses promoted by NBPT averaged 45%.

This difference can be attributed to the degradation of NBPT and diminishment of its inhibitory over the days. As the inhibitory effect fade away, the NH₃ losses began to occur and the cumulative losses from NBPT-treated urea come up, narrowing the differences between treatments. When intense rain incorporated the NBPT-treated urea into the soil right after the losses had started, hence diminishing the NH₃ emissions along the days, the difference to urea losses already occurred is maintained, leading to greater inhibition efficiency. This was

observed in Piracicaba II and Araras trials where rainfalls of 32 and 36 mm, respectively, occurring around the 15 DAF put an end to the NH₃ losses for all treatments.

Higher efficiency of NBPT in reducing NH₃ losses in the first week after fertilizer application have already been reported in the literature. Rawluk, Grant and Racz (2001) observed NBPT efficiencies as great as 96% during the first 5 to 8 DAF, but, throughout the last 12 to 21 DAF, the amount of NH₃ emitted was similar for all treatments reaching a final 37% reduction in loss using NBPT. Engel, Jones e Wallander (2011) also observed that the response to NBPT diminished greatly after a week and higher efficiencies were observed in the firsts 14 DAF.

It is noteworthy that the influence of intensity and distribution of rain on NBPT efficiency is remarkable in systems with a layer of plant residues on soil surface. In such conditions, higher amounts of water is needed to wash the fertilizer through the straw blanket and incorporates the urea into the soil, as related by Cantarella et al. (2008) and Freney et al. (1994). When applied on bare soil, the delay promoted by NBPT by itself allows a great diffusion of urea away from the fertilizer microsite (CHRISTIANSON et al., 1993), leading to small losses and greater efficiencies if compared to trash covered systems (CHIEN; PROCHNOW; CANTARELLA, 2009).

In order to ascertain the best NBPT rate to promote higher reduction in NH₃ losses, a combined regression analysis was performed using data of NH₃ losses accumulated at 30 DAF of the sites presenting homogeneous variance. The results evidenced a remarkable effect of the local and NBPT concentration on the total NH₃ losses (Table 7).

Local		Maan											
Local	0	530	850	1500	2000	Iviean							
-	Accumulated NH ₃ losses at 30 DAF (% of applied N)												
Piracicaba	31.94	27.51	24.97	28.19	23.24	27.17 a							
Piracicaba II	30.84	17.80	11.69	11.14	9.61	16.21 b							
Itirapina	20.40	18.98	16.26	13.52	11.67	16.17 b							
Araras	19.73	11.82	11.16	8.31	7.68	11.74 c							
Iracemápolis	13.44	10.06	7.29	5.63	6.89	8.66 c							
Mean	23.27	17.23	14.28	13.36	11.82	15.99							
$P_{\rm Local} < 0.0001$	$P_{\text{Concentra}}$	_{tion} < 0.0001	$P_{\rm LxC}=0.0$	0782 CV	7(%) = 27.14	HSD = 3.86							

Table 7 – Effect of NBPT rates on cumulative NH₃ losses at 30 days after fertilizer application in five locations¹

¹Means not sharing a letter in the column are significantly different by Tukey's HSD test (P < 0.05)

The cumulative losses observed at 30 DAF were very close to the maximum losses estimated by sigmoidal model, evidencing the applicability of this model for volatilization trials. The total NH₃ loss from untreated urea ranged from 13.4 to 31.9% of applied N in Piracicaba and Iracemápolis, respectively, being the overall NH₃ loss from urea without NBPT averaged in 23.27% of applied N (Table 7). Piracicaba trial also stands out with the greater average cumulative NH₃ loss, with 27% of applied N, followed by Piracicaba II and Itirapina with an average loss of 16%. The lower average emissions occurred in Araras and Iracemápolis, reaching around 10% of the applied N.

Although the particular volatilization ranges for each local (Figure 1), there was no significant interaction between NBPT concentration and local (P<0.05) as shown in Table 7. Therefore, a similar trend of reduction in NH₃ losses as NBPT concentration increases was observed in all five experiments, allowing us to derive a single model to indicate the NBPT concentration that optimize reduction in NH₃ losses.

The linear plateau model was adequate (P=0.0001) in explaining the trend of NH₃ emissions reduction by increasing NBPT rate for five experiments (Figure 2). According to the model, urea presented average NH₃ losses of 23.16% of applied N. For every increment of 100 mg of NBPT, a linear average reduction of 1% of applied N losses were verified until the concentration of 992±238 mg kg⁻¹ of NBPT. Any increment in NBPT concentration above this range did not reflected on substantial reduction of NH₃ losses (Figure 2).



Figure 2 – Effect of NBPT concentration on cumulative NH₃ losses from urea 30 days after fertilizer application in five locations

Watson et al. (2008) obtained 70% of inhibition at a rate of 250 mg kg⁻¹ NBPT with no advantages in increasing NBPT rates above that level in a study performed under controlled conditions. However, under field conditions published studies reveals a potential reduction on volatilization losses as the NBPT concentration increases until a proportion close to the observed in our study. Watson et al. (1994a), for example, observed 93% of inhibition when NBPT rates of 0.1% was used in a five-sites study performed in Ireland during the summer. Likewise, Rawluk, Grant and Rackz (2001) observed a slightly greater inhibition at 0.10 and 0.15% NBPT rates than at the 0.05% rate, while there was no statistical difference in the two higher NBPT rates evaluated.

The model estimates that the treatment of urea with NBPT at 1000 mg kg⁻¹ concentration avoids a loss of around of 10% of the total N applied. Considering the range of losses, it represents an inhibition potential of 43% of the maximum loss compared to urea not treated. In fact, studies under field conditions and mild temperatures, have pointed that when urea is amended with NBPT at a maximum response concentration, around 8% to 24% of total N applied is avoided to be lost (WATSON et al., 1994a; RAWLUK; GRANT; RACZ, 2001; CANTARELLA et al., 2008; CHIEN; PROCHNOW; CANTARELLA, 2009; DAWAR et al., 2011b; SUTER et al., 2013).

4.3 Factors affecting NBPT efficiency in reducing NH₃ losses

The magnitude of NH₃ losses varied more in function of the local of trial than of rate of NBPT (Annex 2). In this study becomes unquestionable clear that the availability of moisture was indispensable for the occurrence of NH₃ loss. However, between the locals were the moisture were not a limitation, there still were verified distinctions either in NBPT efficiency or in the potential of NH₃ loss.

Under field conditions, several factor can influence the NH₃ volatilization and can have contributed to a variable amount of NH₃ emissions in the sites (FRENEY et al., 1992; MACNACK; CHIM; RAUN, 2013; FILLERY; KHIMASHIA, 2016). In purpose to shed light on the main factors that could have contributed to that variation, a principal component analysis were performed with all information available of the experimental sites, except Potirendaba, where the lack of moisture were clearly the absolute factor limiting NH₃ volatilization. The variables that more contributed to explain the variation were combined in four principal components (Table 8). In Each component is a linear association between the variables aiming to capture as much as possible of the variation occurred

Variables	Comp.1	Comp.2	Comp.3	Comp.4
Maximum loss	0.535	0.371	-0.185	0.267
NBPT efficiency	-0.337	0.467	-0.791	
Clay	-0.558		0.220	0.780
Straw cover	-0.267	-0.674	-0.420	-0.158
Temperature	0.466	-0.434	-0.338	0.543
Proportion of variance	0.5233	0.3094	0.1166	0.0507
Cumulative proportion of variance	0.5233	0.8327	0.9493	1.0000

Table 8 - Principal components analysis with the proportion of variance covered by each component

The first component shows that maximum loss and air temperature are strongly positively associated to each other, but have a negative correlation to straw cover and a strongly negative correlation to clay content in soil. In field conditions, a combination of high air temperatures in a soil with low clay content and low straw cover leads to a moisture loss that affect directly the NH₃ losses (FRENEY et al., 1992). It seems, thus, that around 52% of the variation between locals are related to the evaporation of water from soil.

The second component emphasizes the contrast of temperatures and straw content against NBPT efficiency. The combination of the formers can be related to urease activity, and explain around 31% of the variability. Therefore, as shown in Table 8, the first two components combined explain 83% of the total variability in data. These components were put together into a biplot allowing visualizing the correlations between the variables and the trial sites, and the resultant of the two components for each site (Figure 3).

In Figure 3, variables are displayed as arrow-shaped vectors and trial sites are displayed as points. The relationship or correlation among vectors and between vectors and points is positive if their angle is acute, and negative in the case of an obtuse angle. Points that are close together correspond to observations that have similar scores on the components displayed in the plot.

As shown in Figure 3, the lower NH₃ losses were verified in Iracemápolis and Araras, locals where the combination of high clay soil, thick straw layer and low temperatures may have diminished the rate of water evaporation from soil and hence the amount of NH₃ lost. On the other hand, the combination of low clay content, thin straw cover and, in particular, the higher temperatures led to the largest losses observed in Piracicaba experiment (Figure 3).



Figure 3 – Biplot of first two principal components dimensions of principal component analysis of five locations.

Soil texture by itself played an important role in NH₃ losses. Temperature of soil in sandy soils is more susceptible to fluctuations of air temperature than the clay soils (RAWLUK; GRANT; RACZ, 2001). Increments in soil temperature associated with high hydraulic conductivity of coarse soils leads to ascendant water fluxes that move NH₃ upwards near the soil surface where it can easily volatilize (SAN FRANCISCO et al., 2011). Furthermore, higher clay content is directly related to higher exchange capacity which in turn has been shown a negative correlation with NH₃ loss from urea (WATSON et al., 1994b; SAN FRANCISCO et al., 2011; MACNACK; CHIM; RAUN, 2013) due to the adsorption of NH₄⁺ on soil CEC sites.

However, in Piracicaba II and Itirapina trials, the soil texture played no influence in the results obtained, and the influence of straw and temperature was remarkable. In Piracicaba II under low temperatures and low straw content, the greater NBPT efficiency was verified, while in Itirapina in presence of high temperatures and thicker straw layer of the all six trials, the inhibitor efficiency was low (Figure 3).

The high influence of air temperature on NBPT efficiency was noteworthy, being this variables negatively correlated in both components. The component 2 suggests that the high urease activity promoted by straw and warm temperatures, since this enzyme activity increases as the temperature rises (MOYO; KISSEL; CABRERA, 1989; DHARMAKEERTHI; THENABADU, 1996), and by the NBPT degradation under high temperatures resulted in loss of efficiency (BOUWMEESTER; VLEK; STUMPE, 1985; CARMONA; CHRISTIANSON; BYRNES, 1990; SUTER et al., 2011; ENGEL et al., 2013). Notwithstanding the comparative

less favorable conditions to urease activity, Piracicaba II presented the second highest NH₃ loss from urea untreated of the six experiments, what may have been related to the low straw content in that trial, since this variable was very negatively related to the maximum loss.

Indeed, both components points to a strongly negative influence of straw in maximum volatilization loss. This becomes clear in Itirapina trial where, even under high temperatures and very low clay content in soil, the maximum accumulated losses were close to the lower losses of overall trials. In that occasion, the thick straw layer may have acted as an insulate layer, protecting the soil surface from excessive heating and water evaporation, hindering then the NH₃ diffusion up to the top of straw layer and its displacing by wind. In fact, on installation of Itirapina trial, one week after the last rainfall, the interface soil-straw remained wet (what led to the higher straw moisture content near the soil), whilst the top of straw was very dry.

Some authors already observed a reduction of NH₃ losses when urea was surface applied over oat crop residues, if compared to bare soil (SANGOI et al., 2003; DA ROS; AITA; GIACOMINI, 2005). In occasion, it was attributed to the maintenance of soil moisture, which may have favored nitrification and decreased the upward diffusion of ammonia with water evaporation. In addition, Bouwmeester, Vlek and Stumpe (1985) reported that a rapid drought of the surface layer of a clay soil created a barrier between the moist NH₃-enriched soil and the air, decreasing the NH₃ diffusion and hence the losses, what can be compared to the straw effect on this study.

Finally, the high NH₃ loss potential of Piracicaba II was attenuated by the great efficiency of NBPT, whilst the low efficiency of NBPT in Itirapina was compensated by the large amount of straw avoiding water evaporation and NH₃ losses. This reflected in similar average losses in those trials.

5 CONCLUSIONS

The NBPT concentration in urea of 530 mg kg⁻¹ delays the peak of loss in four days whereas concentrations of 1,500 and 2,000 mg kg⁻¹ retard the peak by six days from that occurred for untreated urea.

Linear reduction of NH₃ emissions occurs until 1,000 mg kg⁻¹ of NBPT, which reduces 43% of NH₃ losses as compared to untreated urea. Incrementing NBPT concentration above this range does not reflect on substantial reduction in NH₃ losses.

Doubling the current NBPT concentration in urea (530 mg kg⁻¹) is practicable to reduce NH₃ losses from urea in GCTB systems in Brazil. However, further studies are necessary to define the impact of the N preserved by NBPT on sugarcane yields in an economic context.

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ANNEXES



Annex A – Installation and management of experiments

Experiments installation and management: 1. Straw sampling of the experimental area; 2. Cut of straw around the PVC bases to facilitate the fixation; 3. Fixation of PVC bases into soil;
4. NBPT-amended urea applied inside the PVC base, on the layer of straw; 5. Collect and replacement of the foam discs in the volatilization chamber; 6. View of experiment with collectors already fitted on their respective PCV bases.



Annex B - Extraction and analysis of Nh3 trapped in foams

Extraction and analysis of NH₃ trapped in foams: 1. Addition of deionized water and homogenization with the solution retained in foams; 2. Weighing the beakers containing solution; 3. Weighing the drained foams; 4. Potting the aliquots of the homogenized solution;
5. Aliquots potted and identified; 6. Quantification of N content in aliquots by Flow Injection Analysis.

days, for each treatment in the experiments with significant NH ₃ loss								
Local	Dose	а	a CI(95%)	t0	t0 CI(95%)	b	b CI(95%)	RMSE
Iracemápolis	0	13.4396	0.3694	18.8082	0.3124	2.7106	0.2375	0.3551
	530	9.9865	0.3826	19.9435	0.4179	2.7239	0.3144	0.3194
	850	7.2290	0.5457	20.6078	0.8254	2.9219**	0.5798	0.3911
	1500	6.6419	0.4019	23.9961	0.6133	3.5803	0.3151	0.1264
	2000	7.0110	0.2573	21.0085	0.3862	2.7613	0.2734	0.1830
	0	33.1514	2.1055	11.9300	0.9818	4.0568**	0.7673	2.4820
	530	27.3845	0.3946	16.2547	0.1448	1.6895	0.1351	0.5565
Piracicaba	850	24.8105	0.3481	16.2027	0.1433	1.7384	0.1306	0.4898
	1500	27.9975	0.1271	16.6423	0.0422	1.3114	0.0443	0.1800
	2000	24.1341	1.4417	16.4100	0.7783	3.0410	0.5444	1.5750
	0	19.3316	0.6433	6.8361	0.4324	2.7117	0.4080	1.1840
	530	11.6570	0.2848	10.9185	0.3068	2.5588	0.2632	0.4600
Araras	850	10.9863	0.2405	12.1775	0.2744	2.3478	0.2192	0.3759
	1500	8.3535	0.1396	14.1555	0.2215	2.7228	0.1574	0.1868
	2000	7.6026	0.0914	14.2775	0.1578	2.6627	0.1119	0.1228
	0	29.7320	1.2228	6.1697	0.4888	2.1109	0.4434	2.4790
	530	17.6077	0.3798	12.1793	0.2680	2.2922	0.2147	0.5984
Piracicaba II	850	11.5330	0.1961	12.4412	0.2152	2.3690	0.1680	0.3016
	1500	10.6670	0.2153	12.9166	0.2713	2.7712	0.2033	0.3038
	2000	9.4498	0.3496	14.2266	0.5424	3.6273	0.3780	0.3900
	0	19.9823	0.6246	12.0063	0.3502	1.7914	0.2994	1.0660
Itirapina	530	17.3736	0.6425	15.0562	0.4289	2.0249	0.3192	0.9315
	850	15.1606	0.4075	15.3181	0.2798	1.6432	0.2373	0.6225
	1500	13.5958	0.9917	19.3093	0.9144	3.5372	0.5913	0.7113
	2000	11.2617	1.0725	18.6364	1.2139	3.4695**	0.8034	0.8520

Annex C - Parameters estimated by Boltzmann sigmoidal model

Annex C - Parameters estimated by Boltzmann sigmoidal model for NH3 emissions, along the 30 experimental

 $\text{CI}_{(95\%)}$: Distance of confidence interval at 95% of confidence level

RMSE: Root of mean square of error of model estimation

** Significant by T-test (P < 0.05). All the other parameters were significant by T-test (P < 0.01)

Annex D – Joint analyzes of variance

Annex D.1 –	Summary of joint analy	ysis of variance	performed w	vith the parameters	estimated by	Boltzmann
	sigmoidal model for each	ch block in treat	ments of urea	and urea+NBPT in	Iracemápolis,	Piracicaba,
	Araras, Piracicaba II an	d Itirapina, place	es that presente	ed homogeneous M	lean Square of	Residue

Donomotor	<i>P</i> -value						
rarameter	Local	Dose	Block/Local	Local*Dose			
A _{max} (% of applied N)*	< 0.001	< 0.001	< 0.001	0.809			
T _{max} (days)*	< 0.001	< 0.001	0.420	0.144			
b	0.002	0.109	0.921	0.002			
R _{max} (% of applied N day ⁻¹)*	< 0.001	< 0.001	0.064	0.002			

*Transformed parameter

Annex D.2 – Joint analysis of variance of cumulative NH₃ losses at 30 days after fertilizing of treatments of urea and urea+NBPT in Iracemápolis, Piracicaba, Araras, Piracicaba II and Itirapina, places that presented homogeneous Mean Square of Residue

Factor	DF	Sum Sq	Mean Sq	F value	Pr (>F)	Significance
Local	4	3936.3	984.08	52.25	2.20E-16	***
Dose	4	1636.2	409.05	21.72	4.06E-11	***
Block(Local)	15	2212.0	147.47	7.83	2.59E-09	***
Local*Dose	16	503.4	31.46	1.67	0.0782	•
Residuals	60	1130.0	18.83			
Total	99	9417.9	1590.9			
Signification code:	0 '***'	0.001 '**'	0.01 '**'	0.05 '*'	0.1 '.'	1 ' '