Mini Review

A minireview on what we have learned about urease inhibitors of agricultural interest since mid-2000s

Luzia V. Modolo *, Cristiane J. da-Silva, Débora S. Brandão, Izabel S. Chaves

Departamento de Botânica, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Pres. Antônio Carlos, 6627, Pampulha, Belo Horizonte, MG 31270-901, Brazil

ARTICLE INFO

Article history:
Received 14 February 2018
Revised 14 April 2018
Accepted 15 April 2018
Available online 17 April 2018

Keywords:
Urease inhibitors
Crop production
Pollution mitigation
Urea
Nitrogen fertilizer

ABSTRACT

World population is expected to reach 9.7 billion by 2050, which makes a great challenge the achievement of food security. The use of urease inhibitors in agricultural practices has long been explored as one of the strategies to guarantee food supply in enough amounts. This is due to the fact that urea, one of the most used nitrogen (N) fertilizers worldwide, rapidly undergoes urease-driven hydrolysis on soil surface yielding up to 70% N losses to environment. This review provides with a compilation of what has been done since 2005 with respect to the search for good urease inhibitors of agricultural interests. The potential of synthetic organic molecules, such as phosphoramidates, hydroquinone, quinones, (di)substituted thioureas, benzothiazoles, coumarin and phenolic aldehyde derivatives, and vanadium-hydrazine complexes, together with B, Cu, S, Zn, ammonium thiosulfate, silver nanoparticles, and oxidized charcoal as urease inhibitors was presented from experiments with purified jack bean urease, different soils and/or plant-soil systems. The ability of some urease inhibitors to mitigate formation of greenhouse gases is also discussed.

© 2018 Production and hosting by Elsevier B.V. on behalf of Cairo University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Food production in enough amount and use of better approaches for efficient management of fertilizers are persistent challenges in view of the world population increase [1]. Nitrogen (N) fertilizers are pivotal for crop production as this element is mandatory for plant growth and development. Therefore, application of large amounts of N is a common practice in agriculture [2]. Urea is one of the most used N fertilizer worldwide [3], particularly due to its high N content (46%), relatively low cost per N unit, availability in most markets, high water solubility, low corrosion capacity, compatibility to most fertilizers and high foliar uptake, among others [4]. Despite the wide use of urea as fertilizer, its application on soil raises environmental concerns due to the formation of gaseous
(NH₃, CO₂, N₂O, NO) or ionic (NO₂⁻, NO₃⁻) pollutants from urea hydrolysis, nitrification and denitrification of urea hydrolysis products and NO₃ leaching as well. These events result in increase of greenhouse gas emissions, water pollution and eutrophication and lower N recovery by crops [5–7]. Then, the development of technologies and strategies that allow a more efficient management of N fertilizers and decrease or suppress of their negative effects is desirable for the excellence of the agricultural practices and environmental sustainability.

The use of urease inhibitors is one of the strategies adopted to improve urea performance in agriculture and mitigate urea-driven emission of pollutants [8–11]. Urease is a nickel-dependent enzyme that catalyzes the hydrolysis of urea to two moles of ammonia (NH₃) and one mole of carbon dioxide (CO₂). As a key enzyme for the global N cycle, this hydrolase is widely distributed in nature being found in bacteria, yeasts, fungi, algae, animal waste and plants [12]. A variety of substances have been reported to slow down urease catalytic activity, in which several of them are urea analogs that compete with the natural substrate for the urease active site. If on one hand, urea hydrolysis provides NH₃ that, in turn, is converted to ammonium (NH₄⁺) in soil solution prior to uptake by plants, on the other hand, substantial amounts of N may be lost to atmosphere as NH₃ by volatilization [13,14]. Urease inhibitors are particularly interesting when used in the scope of covering fertilization, in which urea-derived NH₃ formation on soil surface is decreased, favoring, via rain episodes or programmed irrigation, urea movement to deeper soil layer [15]. Then, the control of urease activity in soil may serve as an environmentally friendly alternative to improve N content in soil [16].

Although commercial formulations based on urea and urease inhibitors are available, the efficacy of such inhibitors may vary according to the soil. Indeed, the rate of urea hydrolysis in soils has traditionally been explained by variations in soil physicochemical features such as C and N microbial biomass, surface area, temperature, and pH [6,17,18]. In this context, a broad variety of organic compounds and metal cations (e.g., Hg²⁺, Cd²⁺, Ag⁺, among others) have been investigated for the potential to inhibit ureases with focus on agricultural practices. Therefore, this review brings a compilation of what we have learned since 2005 about urease inhibitors of agricultural interest. It does not include findings related to urease inhibition by plant crude extracts or isolated natural products as we have published a review on this subject in 2015 [9].

**Phosphoramidates**

The N-(butyl) thiophosphoric acid triamide (NBPT; Fig. 1) is the phosphoramidate most known for its use as urease inhibitor in agriculture worldwide. We are giving emphasis to phosphoramidates other than NBPT as the agronomic efficiency of such commercial urease inhibitor is explored in details in another review of this special issue.

The N-(propyl) thiophosphoric triamide (NPPT; Fig. 1), applied together with urea on a Chinese silt (sandy) loam soil under greenhouse condition, slowed down NH₃ volatilization by over 50% in relation to control soil samples during the first 11 days following fertilization [19]. The mixture constituted of 0.05% NPPT and 0.05% NBPT was 23.8% and 28.8% more efficient in mitigating NH₃ volatilization from soil when compared to the single treatments NBPT or NPPT, respectively. Two formulations containing phosphoric acid triamide derivatives (UI1 and UI2) were used on Haplic Phaeozem soil in greenhouse experiments carried out with Avena sativa (oat) [20]. Although it was not clearly disclosed the difference between them, such formulations were likely constituted of the urease inhibitor NPPT. The UI1 improved biomass accumulation (12.3 g dry weight pot⁻¹) and N uptake (339 mg pot⁻¹) in oat panicles as panicles from plants grown under urease-inhibitor-free conditions yielded 9.0 g dry weight pot⁻¹ and 222 mg N pot⁻¹. The N uptake by oat culms from plants under urea + UI1 or urea + UI2 fertilization averaged 231 mg pot⁻¹ while control plant culms accumulated only 150 mg N pot⁻¹ [20]. A commercial formulation named Limus® (25% NPPT + 75% NBPT) was used at 0.12% (w/w related to urea) to fertilize soils from North and Northeast China to grow winter Triticum aestivum (wheat) or summer Zea mays (maize) [21]. Cumulative NH₃ losses reached from 11 to 25% of applied N-urea after two weeks, while soil supplementation with urea plus Limus® decreased the loss by up to 85%. No differences of grain yield was observed between urea-treated and urea plus Limus® soils. These authors also applied Limus® on Fluvial aquic and alluvial soils to grow maize [10]. Limus® treatment promoted, in average, a decrease in cumulative NH₃ losses by 84% compared to urea-treated soils. Additionally, urea plus Limus® improved the apparent N recovery efficiency by 17%. The use of Limus® on the soils tested could reduce by up to 60% the application of N-urea for maize growth and still allowing crop yields as high as those observed from usual farmers’ practice [10].

A urease inhibitor recently introduced to the market, N-(2-nitrophenyl) phosphorionic triamide (2-NPT; Fig. 1), lowered NH₃ volatilization by 26 to 83% from Luvisols (field conditions), causing a 2–3-day delay in the peak of gas emission [22]. As for a field experiment carried out with Lolium perenne (perennial ryegrass) cultivated either in Endofluvic Chernozem or Cambisol, 2-NPT alleviated NH₃ losses by 69–100% when used at concentrations in the range from 0.75 to 1.5 g urea-N kg⁻¹, while urea by itself led to NH₃ volatilization corresponding to up 14% of total N applied [23].

Fourteen phosphoramide derivatives (PADs; Fig. 1) out of 40 compounds synthesized showed higher inhibitory effect on Canavalia ensiformis (jack bean) urease activity than NBPT (IC₅₀ = 100 nM) as they presented concentration necessary to inhibit enzyme activity by 50% (IC₅₀) values ranging from 2 to 63 nM [23]. The most highly active inhibitors (PADs 6k, IC₅₀ = 2 nM; 6p, IC₅₀ = 3 nM and 6f, IC₅₀ = 3.5 nM) were selected for tests in acidic (pH 4.5; Anaya de Alba, Spain), moderate acidic (pH 5.9; Las Planas, Spain) and alkaline (pH 8.5; Mendigorría, Spain) soils. The ability of 6f and 6p to inhibit ureases from moderate acidic soil was comparable to that of NBPT [24]. These phosphoramide derivatives, however, inhibited acidic soil ureases by 65% and alkaline ones by 75% while NBPT inhibited 9% and 45%, respectively. Although 6 k was the most highly active compound in vitro, it showed lower performance on soil ureases than that of 6f or 6p regardless of soil pH. Authors hypothesized that 6 k possesses low stability and fast degradation rate on soil [24].

The extent of the inhibitory effect of phenylphosphorodiimide (PPD; Fig. 1) on urease has been reviewed in 2009 [25]. Since then, the kinetic and thermodynamic behaviors of PPD towards soil ureases were studied at 10, 20 and 30 °C and under waterlogging using Pachic Udic Mollisol (black soil) [26]. The PPD at 50 mg kg⁻¹ dry soil worked as mixed inhibitor as it increased urea Km and decreased ureases Vmax when used at room temperature. The Km and Vmax significantly increased following temperature increment. Soil urease thermodynamic parameters, such as activation energy, enthalpy of activation and temperature coefficients slightly increased upon PPD treatment and increasing temperature when compared to soils devoid of PPD treatment [26]. The PPD treatment led to higher Vmax (ca. 40 nM) and lower Vmax values (ca. 200 mg hydrolyzed urea-N kg⁻¹ dry soil 5 h⁻¹) than those of NBPT treatment up to 30 days of experiment under water-logging. This indicates that PPD is a better urease inhibitor than NBPT in waterlogged soil [27]. The performance of 2% (w/w) PPD as urease inhibitor was also verified in a Calclic Haploxererts soil featuring sandy clay loam texture in the upper (0–28 cm) horizon [28]. The PPD treatment decreased soil urease by ca. 45% during the first two days following application of 120 kg N ha⁻¹ urea. No signifi-
cant effect on N\textsubscript{2}O emissions was observed for soils at 40% and 60% water-filled pore space (WFPS) supplemented with urea plus PPD, although gas emissions increased from 4.5 mg N\textsubscript{2}O-N kg\textsuperscript{-1} d\textsuperscript{-1} (control) to 5.8 mg N\textsubscript{2}O-N kg\textsuperscript{-1} d\textsuperscript{-1} when soils at 80% WFPS received PPD treatment [28].

The substituted phosphoric acid triamide P101/04 at 0.06% (w/w in relation to urea) was used as urease inhibitor in pot experiments devoid of vegetation or with spring wheat grown for 70 days in Cambisol under controlled conditions [29]. The surface application of P101/04 promoted a decrease of N\textsubscript{2}O emission from plant-free soil by 15–46%, regardless of the size of urea granules used. Lower levels (0.16–0.27% of total fertilization) of emitted N\textsubscript{2}O were achieved from the wheat-grown soil [29].

**Hydroquinone and quinones**

It is known that NH\textsubscript{3} formed on soil surface may also be converted to the pollutant N\textsubscript{2}O from either sequential activity of microbial ammonia mono-oxygenase and hydroxylamine oxidoreductase enzymes or from the action of the latter enzyme followed by the activity of denitrifying bacteria [30].

A meta-analysis study with several agricultural soils showed that hydroquinone (HQ; Fig. 2), a urease inhibitor, significantly reduced N\textsubscript{2}O and NO emissions by around 5% [31]. Application of 12 kg N ha\textsuperscript{-1} HQ on an Alluvial soil, in conjunction with 120 kg urea-N ha\textsuperscript{-1}, decreased N\textsubscript{2}O emission by 5% in rice (Oryza sativa) and 7% in wheat systems when compared to the crops grown solely in the presence of 120 kg N ha\textsuperscript{-1} urea [32]. Authors noted, however, that 10% HQ (in relation to urea) + urea contributed to an increment of methane (CH\textsubscript{4}) emission by 12% and then an increase of Global Warming Potential index by 5% [32]. The application of lower amounts of HQ (0.3% in relation to urea) + urea 0.1% in a rizobox system containing 2 kg of sandy loam Belgium soil (classified as luvisol) resulted in a higher number of tillers per rice plants [33]. Furthermore, the association of HQ with dicyandiamide (DCD; nitrification inhibitor) improved rice growth and significantly decreased N\textsubscript{2}O emissions from soil in comparison to urea treatment [33]. The effect of HQ + DCD on N\textsubscript{2}O emission was also analyzed in a soil from a paddy field classified as Typic Hapluga-
do not affect urea ranging from 10 to 30.

Another investigation with black soil showed that temperatures in waterlogged soil was ca. 4°C higher than in non-urea-fertilized soils [35]. The CH4 emission (43.1 ± 4.5 mg kg\(^{-1}\) N\(\cdot\)h\(^{-1}\)) from these same treatments decreased by 35, 19 and 12% and the Global Warming Potential dropped from 99 mg CO2-eq m\(^{-2}\) h\(^{-1}\) to 71.6 and 84 mg CO2-eq m\(^{-2}\) h\(^{-1}\), respectively [33]. In addition, rice grain yield increased by 10%, 18% and 6%, respectively, while the straw weight was improved by 16%, 17% and 8% in comparison to control samples (7.9 and 8.2 t ha\(^{-1}\) for grain yield and straw weight, respectively) [34].

The use of HQ and DCD was investigated in coastal saline Jeru-

salem artichoke bioenergy cropping system maintained in a Flu-

voaquic soil [36]. Urea (300 kg N ha\(^{-1}\)) was used by itself or in conjunction with HQ (30 kg ha\(^{-1}\)) and DCD (9 kg ha\(^{-1}\)) during arti-

choke growing season. The flux of CO2 and N\(_2\)O from soil supple-

mented with urea was 517.9 mg CO2 h\(^{-1}\) and 54.7 mg N\(_2\)O-

N h\(^{-1}\) and decreased by 12 and 16% upon addition of HQ +

DCD, respectively. The net primary production in systems treated

with urea + HQ + DCD increased by 18% in relation to that of

urea-treated ones (18.3 ± 1.36 t C ha\(^{-1}\)). Association of HQ and

DCD with urea yielded a 35% decline in the net ecosystem

exchange of CO2. Likewise, the estimated net greenhouse gas balance and greenhouse gas intensity from Jerusalem artichoke cropping system dropped 37% and 15%, respectively [36].

The efficiency of urease inhibitor HQ was also tested in Pachic

Udic Argiboroll (black soil) at 20% moisture or under waterlogged conditions (3–5 cm water layer). The urea \(K_m\) values towards soil ureases were ca. 36 mM at the first day of soil incubation with HQ and ca. 25 mM 10 days post incubation [27]. In contrast, soil ureases \(V_{\text{max}}\) at 20% moisture increased from 220 mg hydrolyzed urea-N kg\(^{-1}\) dry soil (first day) to 250 mg hydrolyzed urea-N kg\(^{-1}\) dry soil at 10–30 days post-HQ soil treatment while the incre-

ment in waterlogged soil was ca. 40 mg hydrolyzed urea-N kg\(^{-1}\) dry soil during the same period of soil incubation with HQ [27].

Another investigation with black soil showed that temperatures ranging from 10 to 30°C and HQ incubation times up to 20 days do not affect urea \(K_m\) values in soil supplemented with urea + HQ [26]. Temperatures of 20 and 30°C led to significant increment of soil ureases \(V_{\text{max}}\) 10 and 30 days after soil incubation with HQ. Authors also determined that HQ affects soil kinetic parameters much more than it does on soil thermodynamics ones.

Halogen-substituted \(p\)-benzoquinones such as those containing Cl, Br or F atoms has been long recognized as excellent soil urease inhibitors [37]. The mode by which tetrachloro-1,4-benzoquinone and tetrachloro-1,4-benzoquinone affect jack bean urease activity was determined to be as slow-binding inhibition with formation of very stable urease-inhibitor complexes [38]. Tetrachloro-1,4-benzoquinone was more effective than the corresponding ortho-substituted benzoquinone as the urease residual activity reached a plateau in the presence of the former at concentrations much lower (0.29 and 0.59 \(\mu\)M) than those (7.5 and 15 \(\mu\)M) of the latter. The inhibition constants (\(K_i\)) for tetrachloro-1,2-benzoquinone and tetrachloro-1,4-benzoquinone were 2.4 × 10\(^{-10}\) M and 4.5 × 10\(^{-7}\) M, respectively. The interaction between these chloro-

substituted benzoquinones and a Cys residue present in urease active site was responsible for the enzyme inhibition [38].

The inhibition of jack bean urease by 1,4-benzoquinone, 2,5-
dimethyl-1,4-benzoquinone, tetrachloro-1,4-benzoquinone occurs in a concentration-dependent manner, wherein the enzyme-

inhibitor equilibrium was achieved in ca. 10 min [39]. The IC\(_{50}\) values for 1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone and tetrachloro-1,4-benzoquinone (Fig. 2) were 5.5, 50.0 and 0.6 \(\mu\)M, respectively. The residual urease activity was linearly correlated with the number of modified thiols in protein structure. Therefore, arylation of Cys thiol group caused by the quinones tested con-
tributes for the mechanism of enzyme inhibition [39]. Besides ary-
lation of Cys thiol group, 1,4-naphthoquinone (Fig. 2) promotes thiol oxidation. The enzyme inhibition by this benzoquinone is biphasic-type, time- and concentration-dependent with a non-

linear residual activity dependent on thiol modification [39,40].

Indeed, co-crystallization of Sporosarcina pasteurii urease and 1,4-

benzoquinone (41) showed that the enzyme inhibitor covalently binds to the thiol group at \(\alpha\)-Cys322, a highly conserved residue pre-

sent at the mobile flap that controls urea access to urease active site.

### (Di)substituted thioureas

A recent report revealed the potential of benzothiazolyl ureases (BTUs) as urease inhibitors of agronomic interest [42]. An initial in vitro screening performed with 10 mM urea and BTUs at 0.5 mM showed that 51 out of 65 compounds inhibited jack bean urease at different extents [42]. Eight BTUs (11, 12, 14, 19–22, and 37; Fig. 3) were the most potent inhibitors as they negatively affected the ureolytic activity of urease by in the range from 50 to 77%. Such benzothiazolyl ureases function as mixed-type inhibitors exhibiting higher affinity to urease active site than allosteric ones. Based on the equilibrium dissociation constant \(K_i\), BTU 14 was the most efficient mixed inhibitor followed by 11, 22, 19, 37, 20, 21, and 12. Experiments performed with Clayey dystrophic Red Latosol soil supplemented with 0.5 mM BTUs and 72 mM urea showed that compounds 3, 6, 10, 12, 16, 19, and 22 were more efficient than NBPT to inhibit the activity soil ureases. Other 21 BTUs were demonstrated to be as potent as NBPT. Notably, the most efficient BTUs on soil were also found to be more thermostable than NBPT, which makes this class of compounds eligible for further studies towards the development of new urea-based fertilizer formulations [42].

The urease inhibition potential of \(N,N\)-disubstituted thioureas (DSTUs) was evaluated in vitro, using jack bean urease and 100 mM urea [41]. Thirteen thiourea derivatives (DSTUs 1, 3, 4, 9, 13–16, 18–20, 26, and 30; Fig. 3) efficiently inhibited urease activity exhibiting IC\(_{50}\) values (from 8.4 to 20.3 \(\mu\)M) lower than that of standard inhibitor thiourea. These compounds presented Ki values ranging from 8.6 to 19.3 \(\mu\)M and showed mechanisms of action typical of mixed (DSTUs 1, 3, 9,14, 15, 18, and 26), competitive (DSTUs 13 and 30) or non-competitive (DSTU 19) inhibitors [43].

### Benzothiazoles

The inhibitory effect of new benzothiazoles (BZT; Fig. 4) on urease activity was assessed in vitro in reactions containing...
The most effective compounds were 2-phenylbenzothiazole (BZT 1), 2-(4-nitrophenyl) benzothiazole (BZT 2), 2-(4-hydroxyphenyl)benzothiazole (BZT 9), 2-(4-pyridyl)benzothiazole (BZT 15), 2-(3-pyridyl) benzothiazole (BZT 16), 2-(2-carboxyphenyl)benzothiazole (BZT 17) and 2-(1,3-benzodioxol-5-yl)benzothiazole (BZT 18). Among them, BZT 15 was the most active as it inhibited jack bean urease by 55%. The efficiency of hydroxyurea, a reference of inhibitor, averaged 62% [44]. The mechanism by which BZT 15 inhibits jack bean urease is compatible with that of mixed inhibitors that exhibit higher affinity to the active site ($K_i = 1.02 \pm 0.04$ mM) than allosteric ones ($K_i = 3.17 \pm 0.69$ mM) [44].

### Coumarin derivatives

The potential of some coumarinyl pyrazolinyl thiomide (CPTs; Fig. 5) as urease inhibitor was evaluated in vitro using jack bean urease [45]. The derivative bearing an unsubstituted phenyl group (CPT 5n) was the most potent compound exhibiting IC$_{50}$ as low as 0.036 nM from reaction media (90 μL) containing 0.1 U urease, 100 mM urea at pH 8.2 [45]. The presence of an –NO$_2$ at para-position (CPT 5p), an –OH group at para-position (CPT 5q), –Cl and –NO$_2$ at ortho- and meta-positions (CPT 5i) on phenyl ring compromised the anti-ureolytic activity of coumarin derivatives by 17-fold for the former and over 270-fold for CPTs 5i and 5q. The most active compound (CPT 5n) was determined to be a typical non-competitive inhibitor of jack bean urease as increasing concentrations of such coumarin derivative decreased urease activity without significantly affecting urea $K_M$ [45]. Docking studies showed that 5n may form two and one hydrogen bonds with Asp494 and Ala440...
residues present at urease active site, respectively. Hydrogen bond may also be formed between S atom and Asp494.

Phenolic aldehyde derivatives

Four Biginelli adduct were synthesized inspired in the structure of natural phenolic aldehydes namely protocatechuic aldehyde (PA), syringaldehyde (SA) and vanillin (VN) [46]. In vitro assays using jack bean urease (12.5 mU), 10 mM urea and compounds-test at 1.6 mM showed that 2A7 and 2B10 (PA derivatives; Fig. 6) inhibited the ureolytic activity by 94% while enzyme activity inhibition reached 58.6% (in average) when 2A9 (VN derivative) or 2D2 (SA derivative) was added to the reaction medium. These compounds exhibit a mechanism of action typical of mixed inhibitors in which 2A7 was determined to be the most efficient one. The effect on Clayey Dystrophic Red Latosol (oxisol), however, revealed that 2A7 and 2D2 were the most potent against soil ureases as they inhibit the ureolytic activity by 50% when applied at 3.3 mM [46]. This demonstrates that results obtained with purified ureases may not necessary reflect what happens on soil due to its complexity.

Both 2A7 and 2D2 were determined to be more thermal stable than the commercial urease inhibitor NBPT.

Miscellaneous

The use of urea coated with Cu plus Zn on a Malaysian typic paleudult soil greatly improved N uptake by *Pannicum maximum* (Guinea grass) from 12 kg N ha\(^{-1}\) to 137.9 kg N ha\(^{-1}\). Soil supplementation with Cu-coated urea yielded an N uptake by plants of up to 96.7 kg ha\(^{-1}\) [47]. These treatments were shown to slow down urea hydrolysis in comparison with the soil that solely received urea, in which that supplemented with Cu-Zn-coated urea exhibited an increment of pasture production by up to 50% [47]. The use of Cu-B-coated urea in a field study with rice plants cultivated in Typic Albaqualf soil (non-tillage system) reduced the total N-N\(_2\) loss from 47% (urea by itself) to 22% after 96 h of fertilizer application [48]. Likewise, the 1.2% N-NH\(_3\) loss observed in urea-supplied conventional crop system was decreased to 0.3% after 216 h of Cu-B-coated urea application [48]. Rice productivity, however, was not affected by urea coated with Cu plus B. The N loss by NH\(_3\) volatilization was also diminished by urea coated with S or boric acid plus Cu in a field experiment carried out with *Saccharum officinarum* (sugarcane) cultivated in a Brazilian sandy soil [49]. Accumulated N-NH\(_3\) losses from soil treated with acid-boric-Cu-coated urea and S-coated urea were determined to be 2.2 kg ha\(^{-1}\) and 4.6 kg ha\(^{-1}\), respectively, while N-NH\(_3\) loss from soil treated with urea was as much as 9.1 kg ha\(^{-1}\). Therefore, acid-boric-Cu- and S-coated urea mitigated N-NH\(_3\) losses from soil by 75 and 50%, respectively [49]. In 12-month field experiment, the grain yield for maize plants grown in a Brazilian Red Latosol (non-tillage system) containing boric-acid-Cu-coated urea was roughly twice (9.9 kg ha\(^{-1}\)) as much as that of plants grown in the presence of uncoated urea [50]. Application of Cu-B-incorporated urea to Brazilian Hapllic Planosol mitigated total N-N\(_2\) volatilization by 54% compared to commercial urea in an 18-day greenhouse experiment [51]. Also, Cu-B-incorporated urea was up to 36.5% more efficient than Cu-B-coated urea with respect to the ability of inhibiting N-NH\(_3\) loss from soil [51]. The use of a physical mixture constituted of urea, Cu and B postponed the peak of NH\(_3\) volatilization for two days and decreased the total N loss by 18%, compared to commercial urea, in a field experiment carried out with maize cultivated in dystrophic Red Latosols [52]. Nevertheless, the presence of these urease inhibitors did not affect N accumulation in maize grains or stubble. Incorporation of Zn to urea pellets (up to 5 g Zn/kg urea) also efficiently inhibited the activity of red-yellow Oxisol (Typic Hapludox) ureases containing *Megathyrsus maximus* (Guinea grass cv. Mombaça) crop under controlled conditions [53]. Although no significant increment in plants biomass was observed when compared with plants from soil fertilized with urea only, Zn-incorporated urea pellets boosted N-uptake by plants. This is likely due to the ability of Zn to maintain higher levels of N in soil (74% more than that for soils treated with urea only) as a result of its negative effect on NH\(_3\) volatilization [53]. Bench experiments performed for 8 weeks with Malaysian rice soils (Selangor and Chempaka) demonstrated that the use of urea coated with Cu, Zn and Cu + Zn decreased N\(_2\)O emission from soil by 17.6, 21.6 and 29.7%, respectively, in relation to the control [54]. The cumulative N\(_2\)H\(_3\) volatilization from soil for these treatments ranged from 32.1 to 39.6% while soils treated solely with urea emitted 34.7% more N\(_2\)H\(_3\) [54]. These results evidence that the use of Cu-, Zn- or Cu + Zn-coated urea on such Malaysian soils efficiently mitigate the emission of pollutants from urea fertilizer.

Ammonium thiosulfate (ATS) was shown to decrease urease activity in an Italian sandy soil bearing higher pH values and containing relatively lower amount of organic matter [55]. Maximum
urease inhibition (88%) was achieved already three days after application of 100 mg ATS kg^{-1} soil while 25 mg ATS kg^{-1} soil caused a 70% enzyme inhibition. Authors found that ATS by itself or in association with urea did not affect soil microbial biomass pool. On the other hand, a field experiment performed with Canadian clay loam and fine sandy loam soils showed inconsistent results with respect to urease inhibition by ATS [56]. These findings suggest that ATS performance may be affected by the soil type. The complex formed between silver nanoparticles (AgNPs) and jack bean urease was shown to destabilize the hexameric protein structure, a phenomenon than caused loss of ureolytic activity by up 10%, 95% and 100% for urease/AgNPs ratios of 1:1, 1:5 and 1:7, respectively [57]. In this sense, the use of AgNPs as additive in urea-based formulation could be advantageous as such nanoparticles have been also shown to contribute for pest control in agriculture (www.nal.usda.gov/fsrio/research_projects/printresults.php?id = 9104; accessed on Nov 21, 2017).

The dimeric vanadium-hydrazine complexes (DVHCs; Fig. 7) 6c, 10c and 11c act as non-competitive inhibitors and show low phytotoxicity against Lemma aquatilis (duckweed) in comparison to paraquat (known herbicide).

The NH₃ emissions from a 10-cm-surfaced Red-2Yellow Ultisol (under no-tillage) after fertilization with urea coated with oxidized charcoal (produced at 350 °C) were 43% lower than that of soils fertilized with uncoated urea [59]. Additionally, oxidized charcoal delayed the maximum volatilization peak of NH₃ in 24 h, keeping urea-N on soil for longer periods [59]. Similarly, urea coated with 16% oxidized charcoal further reacted with NaOH and urea coated with 39% oxidized charcoal under no alkali treatment also alleviated NH₃ volatilization by 40% from a Hapludalf soil [60]. The N losses to the atmosphere (as NH₃) were also decreased by 12% upon treatment of soils belonging to the subgroups Typic Hapludox, Lamellic Hapludalfs, Aquic Argiudolls and Typic Endoaquolls with urea plus oxidized charcoal [61]. The presence of oxidized charcoal, however, did not change the levels of exchangeable NH₄, NO₃ and NO₂ in the soil in comparison to samples treated with urea only.

Conclusions and future perspectives

Since 2005, several substances, namely phosphorimidates, hydroquinone, benzoquinones, (di)substituted thioureas, benzothiazoles, coumarin derivatives, phenolic aldehyde derivatives, dimeric vanadium-hydrazine complexes, oxidized charcoal, silver nanoparticles have been synthesized and shown to be potential urease inhibitors for use in agriculture. The efficiency of inorganic substances (ammonium thiosulphate, boric acid etc) or metal cations and sulfur on soil ureases was also demonstrated. The ability of urease inhibitors to mitigate the formation of greenhouse gases has been widely investigated focusing on more sustainable agricultural practices. The effect of substituted thioureas, coumarin derivatives and silver nanoparticles on soil ureases deserves investigation since compounds capable of inhibiting jack bean urease may not be active against soil ureases. There is a need for the world market to broaden the offer of urease inhibitors that are effective on distinct types of soil. This is a very challenging task as urea compatibility, efficiency at relatively low concentrations, minimal negative effect on soil microbiota, plant metabolism and human health (if uptaken by crop roots from soil), environmentally friendly capability and prolonged shelf life are criteria that need to be considered for the development of urease inhibitors of agricultural interest.

Conflict of interest

The authors have declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

Acknowledgements

Part of the work described herein was supported by the Conselho Nacional de Pesquisa (CNPq) Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG). LVM is recipient of research fellowship from CNPq.

References

[1] Bueno-Delgado MV, Molina-Martínez JM, Correoso-Campillo R, Pavón-Maríño P. Ecofort: an android application for the optimization of fertilizer cost in fertigation. Comput Electron Agric 2016;121:32–42.
[2] Villalobos F, Fereres E. Principles of agronomy for sustainable agriculture. 1st ed. Springer International Publishing; 2016.
[3] Papangkorn J, Isaraphan C, Phinhongthong S, Opaprakasit M, Opaprakasit P. Controlled-release material for urea fertilizer from polylactic acid. Adv Mat Res 2009;55–57:897–900.
[4] Cantarella H, Trivelin PCO, Conti TLM, Dias PLF, Rossetto R, Marcelino R, et al. Ammonia volatilisation from urea inhibitor-treated urea applied to sugarcane trash blankets. Sci Agric 2008;65:397–401.
[5] Nitrogênio Cantarella H. In: Novais RF, Alvarez VVH, Barros NF, et al., editors. Use of inorganic substances (ammonium thiosulphate, boric acid etc) or metal
[6] Martins MR, Sant’Anna SAC, Zamanc M, Santos RC, Monteiro RC, Alves BJR, et al. Strategies for the use of urease and nitrification inhibitors with urea: impact on N₂O and NH₃ emissions, fertilizer-15N recovery and maize yield in a tropical soil. Agric Ecosystem Environ 2017;247:54–62.
[7] Kiss S, Simih I, Improving efficiency of urea fertilizers by inhibition of soil urease activity. 1st ed. Doedrecht: Kluwer Academic Publishers; 2002.
[8] Modolo LV, Souza AX, Horta LP, Araujo DP, Fátima A. An overview on the potential of natural products as ureases inhibitors. J Adv Res 2015;6:35–44.
[9] Li Q, Cui X, Liu X, Roelcke M, Pasda G, Zerulla W, et al. A new urease-inhibiting formulation decreases ammonia volatilization and improves maize nitrogen utilization in North China Plain. Sci Rep 2017;7:43853.
[10] Mira AB, Cantarella H, Souza-Netto GMJ, Moreira LA, Kamogawa MY, Otro R. Optimizing urease inhibitor usage to reduce ammonia emission following urea application over crop residues. Agric Ecosystem Environ 2017;248:105–12.
[11] Follmer C. Insights into the role and structure of plant ureases. Phytochemistry 2008;69:18–29.
[12] Cameron KC, Di HJ, Moir JL. Nitrogen losses from the soil/plant system: a review. Ann Appl Biol 2013;162:145–73.
[13] Bock BR, Kissel DE. Ammonia volatilisation from urea fertilizers. Muscle Shoals, AL, USA: National Fertilizer Development Center – NFDC; 1988.
[14] Artola E, Cruchaga S, Ariz I, Moran JF, Garnica M, Houdusse F, et al. Effect of N-(n-butyl) triphosphoric triamide on urea metabolism and the assimilation of ammonium by Triticum aestivum L. J Plant Growth Regul 2011;63(1):73–9.
Débora S. Brandão received her BSc. degree in Agronomy and MSc. degree in Crop Production at the Federal University of Minas Gerais (MG, Brazil). She is currently PhD student in Plant Biology under the mentoring of Dr. Luzia V. Modolo. Her research focus is on urease inhibitors and their effects on plant and soil microbiota metabolism.

Izabel S. Chaves was born in 1986. She earned her BSc. degree in Biology in 2009 at the Federal University of Lavras (MG, Brazil). She received her PhD degree in Plant Physiology in 2015 from the Federal University of Viçosa (MG, Brazil). Her research interests include plant physiology and molecular biology as well as the development of novel urease inhibitors for improving plant nitrogen nutrition.