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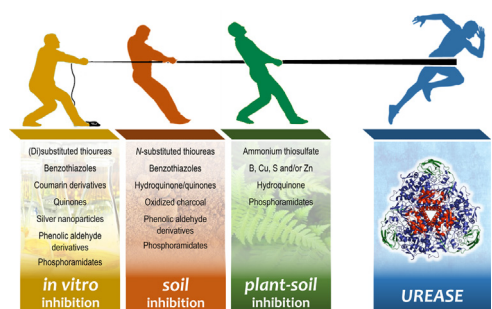
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Mini Review

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

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GRAPHICAL ABSTRACT



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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.

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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

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(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 North-east 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 Fluvio-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) phosphoric triamide (2-NPT; Fig. 1), lowered NH₃ volatilization by 26 to 83% from Luvisol (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 phosphoramidate 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 6 k, 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), moderated acidic (pH 5.9; Las Planas, Spain) and alkaline (pH 8.5; Mendigorria, Spain) soils. The ability of 6f and 6p to inhibit ureases from moderated acidic soil was comparable to that of NBPT [24]. These phosphoramidate 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 phenylphosphorodiamidate (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 K_M and decreased ureases V_{max} when used at room temperature. The K_M and V_{max} 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 K_M (ca. 40 mM) and lower V_{max} 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 Calcic Haploxerepts 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-

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