Original article

Effectiveness of nanoatrazine in post-emergent control of the tolerant weed *Digitaria insularis*

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Abstract

Digitaria insularis (sourgrass) is a monocotyledon weed of difficult control and high invasive behavior. Atrazine is widely applied in the Americas to control weeds in maize culture, but its efficiency against *D. insularis* is limited. The incorporation of atrazine into poly(epsilon-caprolactone) nanocapsules increased the herbicidal activity against susceptible weeds; however, the potential of this nanoformulation to control atrazine-tolerant weeds including *D. insularis* has not yet been tested. Here, we evaluated the post-emergent herbicidal activity of nanoatrazine against *D. insularis* plants during initial developmental stages. The study was carried out in a greenhouse, using pots filled with clay soil. Plants with two or four expanded leaves were treated with conventional or nanoencapsulated atrazine at 50 or 100% of the recommended dosage (1000 or 2000 g · ha^{-1}), followed by the evaluation of physiological, growth, and control parameters of the plants. Compared with conventional herbicide, both dosages of nanoatrazine induced greater and faster inhibition of *D. insularis* photosystem II activity at both developmental stages. Atrazine nanoencapsulation also improved the control of *D. insularis* plants, especially in the stage with two expanded leaves. In addition, nanoatrazine led to higher decreases of dry weight of four-leaved plants than atrazine. The use of the half-dosage of nanoatrazine was equally or more efficient in affecting most of the evaluated parameters than the conventional formulation at full dosage. Overall, these results suggest that the nanoencapsulation of atrazine potentiated its post-emergent herbicidal activity

against *D. insularis* plants at initial developmental stages, favoring the control of this atrazine-tolerant weed.

Kerwords: atrazine, chemical control, nanoherbicide, nanotechnology, sourgrass, tolerant weed control

Introduction

Digitaria insularis (L.) Fedde (sourgrass) is one of the main weeds found in soya bean and maize fields of tropical and subtropical America, occupying an estimated area of 8.2 million hectares in Brazil alone (Lopez-Ovejero *et al*. 2017; Adegas *et al*. 2017). In addition to competing with crops (Gazziero *et al*. 2019), this species is resistant to glyphosate (Melo *et al*. 2019) and has a high dispersion capacity through seeds and rhizomes (Machado *et al*. 2008).

The rotation of herbicides with different mechanisms of action is recommended for the management of *D. insularis* (Silva *et al*. 2017). Acetyl-coenzyme A Carboxylase (ACCase) inhibitors are the major herbicides used for efficient weed control in soya bean culture (Gilo *et al*. 2016). In maize, atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) is commonly used, but its efficiency for the control of *D. insularis* is unsatisfactory and limited to early developmental stages (Gemelli *et al*. 2013; Melo *et al*. 2017). When *D. insularis* plants reach later developmental stages, control is hampered by the lack of herbicides with mechanisms of action that affect annual and perennial grasses (Silva *et al*. 2018); this can occur due to the acquisition of barriers that hinder the herbicide absorption (Marques *et al*. 2011) or by enhanced metabolism (Yu and Powles 2014). The management of *D. insularis* is thus compromised in the rotation of cultures that include maize.

Atrazine is a triazinic selective herbicide that inhibits photosystem II. It can be absorbed by roots or shoots, which allows its application at both pre- and post-emergence (Shaner 2014). It has been banned in Europe, due to its high environmental persistence (half-life of 41–231 days), high toxicity to living organisms, and a propensity for leaching, leading to contamination of ground water (Singh *et al*. 2018). Despite this, atrazine is still widely used to control broad-leaved and some grass weeds in maize, sorghum, and sugarcane cultures in the Americas and Australia (Recker *et al*. 2015). Selectivity occurs due to the metabolism by glutathione S-transferases, which reduce the phytotoxicity of atrazine before it reaches the site of action (Shimabukuro *et al*. 1970).

Applications of nanotechnology in agriculture have been studied in order to increase the efficiency and sustainability of agricultural practices (Iavicoli *et al*. 2017). The development of nano-based products usually consists of the reformulation of active ingredients already registered on the market (Kah *et al*. 2018). In particular, the use of polymeric nanocarrier systems for herbicides has emerged as a promising alternative since they allow a controlled release of the active ingredients, thereby increasing their efficiency towards target organisms and reducing their environmental harm (Pascoli *et al*. 2018; Walker *et al*. 2018).

The incorporation of atrazine into poly(epsilon-caprolactone) (PCL) nanocapsules has been shown to improve weed control when compared with a conventional herbicide, as observed for the post-emergent control of *Amaranthus viridis*, *Bidens pilosa,* and *Brassica juncea* (Oliveira *et al*. 2015a; Sousa *et al*. 2018). Other advantages of nanoatrazine include improved stability, slow release of the herbicide, application of lower dosages, and reduced toxicity to non-target organisms (Grillo *et al*. 2012; Oliveira *et al*. 2015a; Sousa *et al*. 2018). The use of nanoherbicides may also be related to an increased absorption of the active ingredient (Anton *et al*. 2008). A recent mechanistic study showed that nanoatrazine enters mustard plants through leaf stomata (Bombo *et al*. 2019). However, it has not yet been determined if atrazine nanoencapsulation would avoid mechanisms of tolerance.

Thereat, the post-emergent herbicidal activity of nanoatrazine was evaluated against *D. insularis* plants at two initial developmental stages and compared it to a conventional atrazine formulation. It has been hypothesized that atrazine nanoencapsulation would improve the post-emergence control of this atrazine-tolerant weed.

Materials and Methods

The experiment was carried out in a greenhouse of the State University of Londrina using a completely randomized design with five replicates. The experimental units were 1 l pots (10.5 cm high, 9.5 cm lower diameter, 14 cm upper diameter) filled with soil (77.8% clay) collected from an herbicide-free area. This soil with high clay content is typical for northern Paraná, and had the following chemical characteristics: pH ($CaCl_2$) – 4.83; organic matter – 28.2 g dm^{-3}; P – 7.63 mg · dm^{-3}; K – 0.65 $cmol_c$ · dm^{-3}; Na – 0.0 $cmol_c$ · dm^{-3}; Ca – 3.96 $cmol_c$ · dm^{-3}; Mg – 1.80 $cmol_c$ · dm^{-3}; sum of bases – 6.41 $cmol_c$ · dm^{-3}; cation exchange capacity at pH 7.0 (CEC) – 11.0 $cmol_c$ · dm^{-3}; and base saturation (BS) – 58.2%. BS was calculated as Equation 1:

$$BS = 100 \times (K + Ca + Mg)/CEC.$$

Seeds of *D. insularis* (L.) Fedde (sourgrass) with a history of glyphosate resistance were collected from a field at Londrina, Paraná, Brazil (23°20'24.7"S 51°12'36.6"W). Seeds were sown directly into the soil-filled pots and after emergence five seedlings of homogenous size were kept per pot. Two simultaneous post-emergent experiments were performed using plants at different developmental stages: two expanded leaves (stage 1) or four expanded leaves (stage 2). Plants reached stages 1 and 2 at 14 or 21 days after emergence, respectively.

The plants were submitted to the following treatments: atrazine-loaded PCL nanocapsules applied at doses of 1000 g a.i. · ha^{-1} (NC+ATZ 1) or 2000 g a.i. · ha^{-1} (NC+ATZ 2), a conventional formulation of atrazine at 1000 g a.i. · ha^{-1} (ATZ 1) or 2000 g a.i. · ha^{-1} (ATZ 2) and control (without herbicide application). The highest dosage (2000 g a.i. · ha^{-1}) is commonly recommended for weed control in maize culture, and the half-dosage was tested to determine if nanoencapsulation potentiated the herbicidal activity. Atrazine-loaded PCL nanocapsules were prepared by the nanoprecipitation method as described by Grillo *et al*. (2012), resulting in a suspension with 1 mg · ml^{-1} of the herbicide. Briefly, the organic phase was composed of 100 mg of PCL, 40 mg of SPAN 60 (sorbitan monostearate surfactant), 200 mg of myritol, and 10 mg of atrazine dissolved in 30 ml of acetone. The organic phase was added to the aqueous phase (Tween 80 2 mg · ml^{-1}) and the mixture was kept under magnetic stirring for 20 minutes. Finally, the formulation had its volume reduced to 10 ml by rotoevaporation. The resulting nanocapsules were characterized using the methodologies described by Grillo *et al*. (2012). They had a hydrodynamic size of 240 ± 4 nm, a polydispersity index of 0.041 ± 0.05, a zeta potential of 30 ± –2 mV, and an encapsulation efficiency of 94%, similar to the characteristics previously reported for this nanoformulation (Grillo *et al*. 2012). The conventional atrazine formulation used in this study was Gesaprim ® 500 CG (500 g a.i. · ml^{-1}, SC, Syngenta).

The formulations were applied early in the morning (between 7:00 and 8:00 a.m.) with a hand sprayer, using a volume of 5.1 ml per pot. The required amount of each formulation was calculated according to the desired dosage, the pot area, and the concentration of atrazine in the formulation, which was diluted in water prior to application. For both experiments, the treatments were performed on the same day (average temperature 23.5°C, relative humidity 81%). Throughout the experiments, the pots were irrigated with water on alternate days. The experimental units were irrigated on the day before and after the application.

Chlorophyll *a* fluorescence parameters were measured using a portable fluorometer OS1p (Opti-Sciences, Hudson, USA) at 8, 24, 48, 72, and 96 hours after application (HAA). The leaves were dark-adapted for 20 min using FL-DC clips and the minimum fluorescence

(F_0) was measured using a weak modulated light (10% intensity) for 0.1 s. Then, the leaves were exposed to a light saturating pulse (8,250 μmol m^{-2} s^{-1}) for 0.8 s to measure the maximum fluorescence (F_m). The variable fluorescence (F_v) was calculated as the difference between F_m and F_0. The maximum quantum yield of photosystem II was determined as the F_v/F_m ratio (Baker 2008). The optimal values of F_v/F_m are near 0.8; thus, in this study, lowered values indicated the inhibitory action of atrazine in photosystem II.

To estimate the relative electron transport rate of photosystem II (rETR), leaves exposed to natural photosynthetically active radiation (PAR) were used. The basal fluorescence (F') and the maximum fluorescence (F_m') of light-adapted leaves were determined before and after exposure to the light saturating pulse, respectively, and ΔF was calculated as the difference between F_m' and F'. rETR was calculated as Equation 2 (Baker 2008):

$$\text{rETR} = \Delta F/F_m' \times \text{PAR} \times 0.5 \times 0.84.$$

The evaluation of plant control (%) was performed 7 days after application (DAA). A scale from zero (plants without any symptoms) to 100 (complete death of the plants) was used. The plants were then harvested and washed in water with care to avoid root loss. They were kept at 60ºC until they reached a constant weight to measure the dry matter of root and shoot.

Data for each experiment were analysed separately. The variables F_v/F_m and percentage of control were previously transformed to arc sin √x to achieve the assumptions of normality of errors and homogeneity of variances. Data were submitted to analysis of variance (ANOVA) and, when significant, the means were compared by Tukey's test ($p < 0.05$), using software R.

Results

Experiment with *D. insularis* plants at stage 1 (two expanded leaves)

At the standard dosage, nanoatrazine induced higher and faster reductions of maximum photosystem II (PSII) activity of *D. insularis* plants at stage 1 than conventional atrazine (Fig. 1A). At 8 HAA, nanoatrazine (2000 g a.i · ha^{-1}) inhibited F_v/F_m by nearly 70% more than the controls, whereas conventional atrazine at the same dosage led to a similar inhibition at 72 HAA. Even when applied at half-dosage, nanoatrazine was more efficient than conventional atrazine at full dosage in reducing F_v/F_m of *D. insularis* plants, with the exception of 48 HAA, when nanoatrazine (1000 g a.i. · ha^{-1}) led to the same inhibition of PSII activity as conventional atrazine (2000 g a.i. · ha^{-1}). In contrast, the dilution of conventional atrazine

compromised its inhibitory effect on PSII, as indicated by the high F_v/F_m values measured in plants treated with this formulation. At the last measurement (96 HAA), F_v/F_m was reduced by 94 and 100% with nanoatrazine and by 38 and 52% with conventional atrazine, at 1000 and 2000 g a.i. · ha^{-1}, respectively.

Fig. 1

At 24 HAA, rETR was lower in *D. insularis* plants treated with conventional atrazine at 2000 g a.i. · ha^{-1} or with both dosages of nanoatrazine than in plants treated with the half-dosage of conventional atrazine (Fig. 1B). From 24 HAA onwards, rETR could not be measured in atrazine-treated plants at stage 1 due to the low fluorescence values of light-adapted leaves.

Nanoencapsulation also improved the control of *D. insularis* plants by atrazine (Fig. 2A). At 7 DAA, nanoatrazine (2000 g a.i. · ha^{-1}) induced a control of 97%, whereas the control provided by conventional atrazine at the same dosage was of only 57%. For physiological parameters, the control induced by nanoatrazine (1000 g a.i. · ha^{-1}) did not differ from that provided by double the amount of conventional atrazine. Moreover, the application of the lowest dose of conventional atrazine did not result in effective control of *D. insularis* plants, since it did not differ from that of plants without herbicide treatment. All treatments with atrazine formulations, however, decreased root, shoot, and total dry weights of *D. insularis* plants to the same extent (Fig. 2B).

Fig. 2

Experiment with *D. insularis* plants at stage 2 (four expanded leaves)

At 8, 24, and 48 HAA, both dosages of nanoatrazine led to greater reductions of maximum PSII activity than the conventional formulation at 2000 g a.i. · ha^{-1} (Fig. 3A). At 72 HAA, these treatments induced the same inhibition of F_v/F_m. However, at the last evaluation, nanoatrazine (2000 g a.i. · ha^{-1}) almost eliminated the PSII activity of *D. insularis* leaves, whereas conventional atrazine (2000 g a.i. · ha^{-1}) and nanoatrazine (1000 g a.i. · ha^{-1}) led to similar reductions of F_v/F_m (around 50%).

The effects of the formulations on rETR followed the same trend observed in F_v/F_m analysis (Fig. 3B). The application of both dosages of nanoatrazine led to the lowest values of rETR at 24 and 48 HAA. At 72 HAA, the inhibitory effect of conventional atrazine (2000 g

a.i. · ha^{-1}) on rETR matched those of nanoatrazine, but conventional atrazine (1000 g a.i. · ha^{-1}) was the least efficient.

Fig. 3

The application of both dosages of nanoatrazine and the highest dose of conventional atrazine resulted in a similar control of *D. insularis* plants at stage 2, although nanoatrazine (2000 g a.i. · ha^{-1}) numerically led to higher control percentages (around 50%) (Fig. 4A). Again, the control provided by the lowest dosage of conventional atrazine did not differ from that of untreated plants.

In contrast to the results observed with *D. insularis* plants at stage 1, the formulations differed in their effects on the dry weight of plants at stage 2 (Fig. 4B). Nanoatrazine (2000 g a.i. · ha^{-1}) was the only treatment that induced a significant decrease of shoot dry weight compared to control plants. Regarding root dry weight, both nanoatrazine concentrations and conventional atrazine (2000 g a.i. · ha^{-1}) negatively affected this parameter to the same extent, whereas the root dry weight of plants treated with conventional atrazine (1000 g a.i. · ha^{-1}) did not differ from that of untreated plants. When considering the total dry weight of *D. insularis* plants, only nanoatrazine (regardless of the dosage) significantly reduced this parameter compared with the control.

Fig. 4

Discussion

The analysis of physiological, growth, and control parameters of *D. insularis* plants demonstrated that atrazine nanoencapsulation improved the post-emergent activity of the herbicide against this weed species. When the formulations were applied at the standard dosage, nanoatrazine induced stronger effects than conventional atrazine on the majority of the evaluated parameters of two- and four-leaved *D. insularis* plants. Moreover, the half-dosage of nanoatrazine was more efficient than, or as efficient as, conventional atrazine at full dosage in affecting most parameters of plants at both developmental stages. In contrast, the dilution to 1000 g a.i. · $ha^{-1}$of conventional atrazine greatly compromised its herbicidal activity. Thus, the incorporation of atrazine into PCL nanocapsules emerges as a promising alternative to increase the efficiency of the post-emergent control of *D. insularis* plants at initial stages of development. Although atrazine nanoencapsulation has been previously

reported to potentiate the herbicidal activity against susceptible plants (Oliveira *et al*. 2015a; Sousa *et al*. 2018), the present study is the first to our knowledge to demonstrate the potential of a nano-herbicide in improving the control of a tolerant weed.

The application of atrazine has been shown to have a low efficiency in the post-emergent control of *D. insularis* and related species. Melo *et al*. (2017) reported a control of 43.7% 7 days after treating *D. insularis* plants at the one to two tiller stage with 3000 g atrazine · ha^{-1}. In a similar study, Dan *et al*. (2011) observed a control of 18.8 and 15.1% 7 days after treating 14- and 28-day-old *D. horizontalis* plants, respectively, with conventional atrazine at 4000 g a.i. · ha^{-1}. Here, the application of nanoatrazine at 2000 g a.i. · ha^{-1} resulted in a control of 97 and 50% of *D. insularis* plants at the two or four leaf stages, respectively. Conventional atrazine at the same dosage, however, led to a less efficient control of plants at these stages (62 and 28%, respectively).

Our results corroborate previous studies reporting that atrazine tolerance in grasses increases as the plant develops (Gemelli *et al*. 2013; Melo *et al*. 2017). This behavior is associated with decreased herbicide absorption due to characteristics of plant tissues and an increased action of enzymes that conjugate the herbicide (Shimabukuro *et al*. 1970; Catâneo *et al*. 2002). Furthermore, these traits have a higher capacity to recover from the damage caused by application (Marques *et al*. 2011). Although nanoatrazine potentiated the control of four-leaved *D. insularis* plants, the efficiency was not completely satisfactory. Notwithstanding, the higher efficiency of nanoatrazine in inhibiting photosynthetic activity reflected in a stronger reduction of the dry weight of four-leaved plants by this formulation, which could limit the competitive capacity of *D. insularis* in a crop field.

Absorption and metabolism are the main processes that may affect plant sensitivity to herbicides (Monquero *et al*. 2004). Bombo *et al*. (2019) recently reported that atrazine-containing PCL nanocapsules adhered to leaf surfaces and entered the mesophyll through stomatal apertures in *Brassica juncea* leaves. In addition, the incorporation of atrazine into PCL nanocapsules led to a sustained release of the herbicide over the days after application. Thus, it might be hypothesized that the improved efficiency of nanoatrazine against *D. insularis* may be related to a greater leaf uptake or a longer-term presence of the herbicide in plant metabolism, thus surpassing the tolerance mechanisms of the weed.

Despite the improved control of *D. insularis* plants at initial stages, the post-emergent treatment with atrazine-loaded PCL nanocapsules led only to transient effects on photosynthetic and oxidative stress parameters of 14-day-old corn plants (Oliveira *et al*. 2015b). The plants were fully recovered 4 days after application of the nanoformulation, and

their growth remained unaffected when evaluated 7 days after treatment, thereby suggesting that the atrazine selectivity in corn plants was maintained after nanoencapsulation (Oliveira *et al*. 2015b). In addition, Grillo *et al*. (2012) reported that atrazine-loaded PCL nanocapsules had less genotoxic effects against non-target plants than conventional atrazine. Preisler *et al*. (2019) recently demonstrated that the long-term residual effect of atrazine on soya bean plants was not increased by nanoencapsulation.

Overall, our results may have important implications on weed management practices, as atrazine is still one of the most frequently used herbicide in corn culture of many countries (Recker *et al*. 2015) and it has low efficiency in the control of *D. insularis*, one the most relevant summer weeds in tropical and subtropical areas (Lopez-Ovejero *et al*. 2017). The use of PCL nanocapsules as carrier systems for atrazine may therefore improve the management of such weeds. Further studies under field conditions are necessary to validate these results, as well as the evaluation of the activity of nanoatrazine against other weed species and non-target organisms.

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Figure Legends

Fig. 1. Physiological parameters of *Digitaria insularis* plants at stage 1 (two expanded leaves). A – maximum quantum yield of photosystem II (F_v/F_m) at 8, 24, 48, 72 and 96 h after application (HAA) and B – relative electron transport rate of photosystem II (rETR) at 24 HAA, measured in leaves treated with water (Control – CT), conventional atrazine at 1000 (ATZ 1) or 2000 g a.i. · ha^{-1} (ATZ 2), and nanoatrazine at 1000 (NC+ATZ 1) or 2000 g a.i. · ha^{-1} (NC+ATZ 2). Data are mean ± standard error (n = 10). The same letters above the bars indicate means that do not differ according to Tukey's test ($p < 0.05$)

Fig. 2. A – percentage control and B – dry weight control of *Digitaria insularis* plants at stage 1 (two expanded leaves) 7 days after treatment with water (Control – CT), conventional atrazine at 1000 (ATZ 1) or 2000 g a.i. · ha^{-1} (ATZ 2), and nanoatrazine at 1000 (NC+ATZ 1) or 2000 g a.i. · ha^{-1} (NC+ATZ 2). Data are mean ± standard error (n = 5). The same letters above the bars indicate means that do not differ according to Tukey's test ($p < 0.05$). (C) Images of experimental units representative of each treatment

Fig. 3. Physiological parameters of *Digitaria insularis* plants at stage 2 (four expanded leaves). A – maximum quantum yield of photosystem II (F_v/F_m) at 8, 24, 48, 72 and 96 h after application (HAA) and B – relative electron transport rate of photosystem II (rETR) at 24, 48, 72 HAA, measured in leaves treated with water (Control – CT), conventional atrazine at 1000 (ATZ 1) or 2000 g a.i. · ha^{-1} (ATZ 2), and nanoatrazine at 1000 (NC+ATZ 1) or 2000 g a.i. · ha^{-1} (NC+ATZ 2). Data are mean ± standard error (n = 10). The same letters above the bars indicate means that do not differ according to Tukey's test ($p < 0.05$)

Fig. 4. A – percentage control and B – dry weight control of *Digitaria insularis* plants at stage 2 (four expanded leaves) 7 days after treatment with water (Control – CT), conventional

atrazine at 1000 (ATZ 1) or 2000 g a.i. · ha^{-1} (ATZ 2), and nanoatrazine at 1000 (NC+ATZ 1) or 2000 g a.i. · ha^{-1} (NC+ATZ 2). Data are mean ± standard error (n = 5). The same letters above the bars indicate means that do not differ according to Tukey's test ($p < 0.05$). (C) Images of experimental units representative of each treatment