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Original article

Pre-emergence application of (thio)urea analogues compromises the development of the weed species *Bidens pilosa*, *Urochloa brizantha*, and *Urochloa decumbens*

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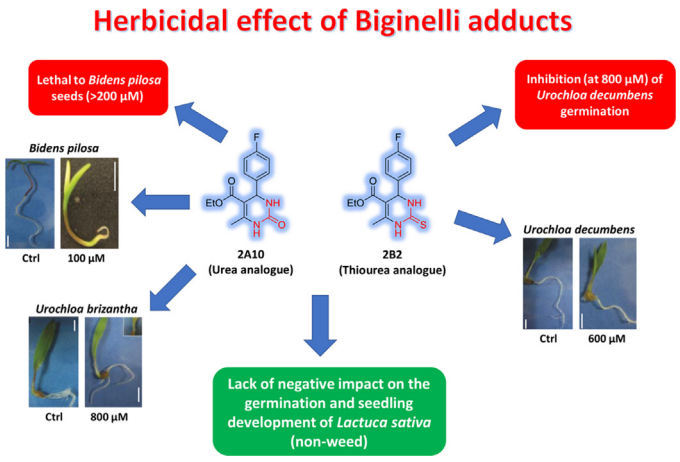
HIGHLIGHTS

- (Thio)urea derivatives are promising pre-emergence herbicides.
- The urea derivative 2A10 strikingly inhibits the germination of *B. pilosa* seeds.
- The thiourea derivative 2B2 impairs the growth of lateral roots in *U. decumbens*.
- 2A10 and 2B2 are either innocuous or beneficial to the non-weed *L. sativa*.

GRAPHICAL ABSTRACT



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ABSTRACT

Invasive species (weeds) contribute to great losses in crop productivity, and one of the strategies for controlling their distribution in the field involves the use of herbicides. However, the development of new formulations for the control of weeds is challenged by environmental issues, increases in the resistance of weeds to herbicides, and poor selectivity of herbicides towards invasive species. Here, by using pre-emergence experiments, we assessed the phytotoxicity of two (thio)urea analogues (2A10 and 2B2) against the weed species *Bidens pilosa* (a dicot), *Urochloa brizantha* and *Urochloa decumbens* (monocots). Similar to diuron (400 μM), which is a commercial urea analogue herbicide, the urea analogue 2A10 (>200 μM) was lethal to *B. pilosa*. Although 2A10 failed to disrupt the germination of *U. brizantha* seeds, this compound (≥600 μM) inhibited the accumulation of chlorophyll *a* and *b* and carotenoids and resulted in the development of seedlings that presented relatively short roots and small, chlorotic leaves. Moreover, the thiourea analogue 2B2 (≥600 μM) reduced the germination percentage of *U. decumbens* seeds and delayed their germination, and at a concentration of 800 μM, this analogue impaired root

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growth and blocked the formation of lateral roots. The presence of an oxygen atom in the urea moiety of the 2A10 structure is critical for its marked activity against *B. pilosa* seeds, as 2B2 bears a sulphur atom instead and marginally inhibits seed germination. Neither 2A10 nor 2B2 was toxic to the non-weed species *Lactuca sativa* (lettuce; a dicot), and the latter even exerted beneficial effects by stimulating leaf expansion. Therefore, the evaluated (thio)urea analogues are promising for the design and development of new phytotoxic compounds for the pre-emergent control of the spread of *B. pilosa* (2A10) or the post-emergence control of *U. brizantha* (2A10) and *U. decumbens* (2B2).

Introduction

The invasion of weedy plant species can negatively impact crop production, civil construction and biodiversity [1]. Specifically, weed species can take up as much as 40% of soil nutrients, which could compromise the production of food crops [2]. Indeed, the increasing demand for food and the restoration of degraded biomes require the establishment of efficient strategies for controlling weed proliferation-triggered crop losses [3], such as hand pulling, tillage, mowing and the use of fire/heat or herbicides [4]. The use of herbicides is considered one of the most efficient options, particularly in cases in which the nuisance species affecting crop production is widely spread and in which mechanical control might severely damage the environment [5]. In fact, the use of selective herbicides has become the most widely used strategy for controlling weed distribution in ecosystems in the USA [6]. These types of agrochemicals can selectively kill 90 to 99% of weed species [7], especially when applied at pre-emergence [8]. However, increases in the resistance of plants to herbicides and safety concerns with respect to human health and the environment have become great challenges that need to be overcome [9]. More than 214 weed species have been found to exhibit resistance to herbicides [10], and Brazil, with 31 weed species that are resistant to herbicides, is ranked eighth based on the number of herbicide-resistant weed species [11]. *Bidens pilosa* [12–14], *Digitaria insularis* [11], *Euphorbia heterophylla* [15], and *Lolium multiflorum* [11,16–18] are among the weed species with known resistance to pesticides.

Efforts attempting to develop novel, environmentally friendly and more efficient herbicides have increased [19], and as a result, substances that can be degraded by soil microbiota and/or are selective to weed species have become interesting candidates for the mitigation of weed propagation [19]. Biginelli adducts, which are substances obtained from the cyclocondensation of acetoacetic esters, aromatic aldehydes and (thio)urea [20,21], have the ability to inhibit both cancer cell proliferation and fungal growth and control blood pressure {reviewed by [22,23]}. Notably, the literature does not describe the potential of Biginelli adducts as herbicides, even though they are structurally related to urea, similar to the widely used herbicide diuron (Fig. 1). This study investigated the potential of the Biginelli adducts 2A10 and 2B2 (Fig. 1), which are structurally related to urea and thiourea, respectively, to inhibit the development of the dicot weed *B. pilosa* (hairy beggarticks) and the monocot weeds *Urochloa brizantha* (formerly *Brachiaria brizantha*; beard grass) and *Urochloa decumbens* (formerly *Brachiaria decumbens*; signal grass) at the pre-emergence stage. The dicot *Lactuca sativa* (lettuce) was also used to assess the selectivity of the compound towards weeds.

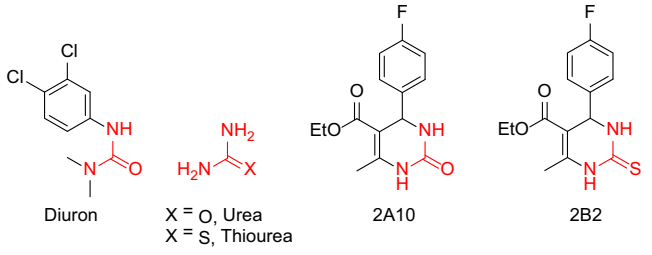

Fig. 1. Structure of urea and thiourea analogues evaluated for the ability to impair weed growth. Urea and thiourea moieties are highlighted in red color.

Material and methods

Preparation of the Biginelli adducts

Biginelli adducts 2A10 and 2B2 were prepared according to the methodology developed by our research group [24].

Seed germination

Seeds of iceberg lettuce (*L. sativa* cv. Diva) sold by Topseed Garden were obtained from a local market (Belo Horizonte, MG, Brazil), seeds of *B. pilosa* were obtained from the Brazilian Agricultural Research Corporation (EMBRAPA) Milho e Sorgo (Sete Lagoas, MG, Brazil), and seeds of *U. brizantha* and *U. decumbens* were kindly provided by the seed supplier Sementes Faria (Belo Horizonte, MG, Brazil).

Twenty seeds of each species were transferred to Petri dishes containing Whatman paper imbibed with 4 mL of 2A10 or 2B2 solutions at concentrations of 0, 50, 100, 200 or 400 μM. The Petri dishes were maintained in a bio-oxygen demand chamber at a temperature of 25 ± 3 °C and under a 12-h photoperiod, and the germination of the seeds was monitored daily for 15 (*Urochloa* spp.) or 10 days (*B. pilosa* and *L. sativa*). The experiments were performed with five replicates, and 400 μM diuron was used as the reference herbicide. At the end of the experiments, the viability and vigour of the non-germinated seeds were determined using the tetrazolium test [25].

The results are expressed as the germination percentage, germination speed index (GSI) [26], germination rate of 50% of the total seed population (T50) [27], primary root length and leaf area.

Chloroplast pigments and biomass accumulation

The leaves (0.02 g) were harvested at the end of the experiments and treated with dimethyl sulfoxide (DMSO) (5 mL) for 24 h (*B. pilosa* and *L. sativa*) or 48 h (*Urochloa* spp.) in the dark for the extraction of chloroplast pigments [28]. The absorbance (Abs) of the supernatants was measured at 480, 649 and 665 nm, and the total chlorophyll and carotenoid amounts were determined using the following equations:

$$\text{Chlorophyll } a(\alpha) = (12.19 \times \text{Abs}_{665\text{nm}}) - (3.45 \times \text{Abs}_{649\text{nm}})$$

$$\text{Chlorophyll } b(\beta) = (21.99 \times \text{Abs}_{649\text{nm}}) - (5.32 \times \text{Abs}_{665\text{nm}})$$

$$\text{Total carotenoids} = [(1000 \times \text{Abs}_{480\text{nm}}) - (2.86 \times \alpha) - (70.16 \times \beta)]/220$$

The results of the chloroplast pigment accumulation are presented as milligrams per gram of fresh weight. In addition, seedlings were dried at 60 °C in a forced-air circulation oven until a constant weight was reached to estimate the biomass accumulation, and the results are presented as the percentage of dry weight in relation to the fresh weight.

Statistical analyses

A randomized experimental design with at least five biological replicates was used. The data were assessed via two-way analysis of variance (ANOVA), and the mean values were compared using Tukey's and Dunnett's tests ($P < 0.05$) [29]. These analyses were performed using R software (www.r-project.org).

Results

Effects of 2A10 and 2B2 on the germination of weed seeds

The tetrazolium test was performed on the non-germinated seeds at the end of the germination experiment to calculate the actual percentage of germinated seeds. This was performed because the viability of seeds in a population varies according to the plant species [30]. Thirty to 35% of the *U. brizantha* and *U. decumbens* seeds that failed to germinate were non-viable, while the percentage of inviable seeds of *B. pilosa* and *L. sativa* ranged from 20 to 30% and from 10 to 15%, respectively, regardless of the treatment.

The treatment with compound 2A10 at concentrations equal to or greater than 400 μM completely abolished the germination of *B. pilosa* seeds, whereas concentrations of 100 or 200 μM resulted in an average inhibition that was 20.7% lower than that of the negative control (0 μM; $P < 0.0001$). Diuron (400 μM), which was used as the reference herbicide, also completely inhibited the germination of *B. pilosa* seeds (Table 1). Thus, 2A10 was used at a concentration ranging from 50 to 200 μM in all subsequent pre-emergence experiments performed with *B. pilosa*. Compared with that of the negative control, the T50 of *B. pilosa* seeds in the presence of 50 μM 2A10 or 200 μM 2A10 resulted in 1.8- and 3.4-day delays in root protrusion, respectively (Table 1). Similarly, compared with the negative control, 600 and 800 μM 2B2 inhibited the germination of *B. pilosa* seeds by 60% ($P < 0.0001$), although 2B2 was 40% less effective than diuron ($P < 0.001$). The GSI for *B. pilosa* seeds treated with 600 or 800 μM 2B2 was 70% lower than that of the negative control seeds ($P < 0.0001$), with no effect on the T50 (Table 1). Compared with that of the negative control seeds, the germination percentage of the *U. decumbens* seeds treated with 2A10 and 2B2 decreased, while diuron had no effect (Table 1). In fact, treatment of *U. decumbens* seeds with ≥600 μM 2A10 inhibited germination by 39–50% ($P < 0.008$), whereas compared with the negative control, 800 μM 2B2 reduced germination by 31.5% ($P < 0.005$) and reduced the GSI by 51.7% ($P < 0.001$). At concentrations up to 800 μM, none of the compounds, including diuron, affected the germination of *U. brizantha* and *L. sativa* seeds (Table 1; $P > 0.05$).

Effects of 2A10 and 2B2 on the development of weed seedlings

The roots of *U. brizantha* seedlings originating from seeds germinated in the presence of 400–600 μM or 800 μM 2A10 were found to be 23% and 54% shorter, respectively, than those of the negative control seedlings (Table 2; $P < 0.001$). The presence of 600 μM 2B2 inhibited root elongation by 31%, whereas compared with that in response to the negative control, the root growth in response to this thiourea derivative at concentrations of 400 and 800 μM increased by 71.5% and 31%, respectively. The leaf area of *U. brizantha* seedlings treated with 400 and 600 μM 2A10 (or 2B2) or 800 μM 2A10 was 32% and 55% smaller than that of the negative control seedlings (Table 2; $P < 0.0001$). Diuron (positive control) was more efficient than 2A10 and 2B2 with respect to *U.*

Table 1
Effects of compounds 2A10 and 2B2 or by diuron (control) on the germination of *Bidens pilosa* (*Bp*), *Urochloa brizantha* (*Ub*), *Urochloa decumbens* (*Ud*), and *Lactuca sativa* (*Ls*) seeds.

	Compound 2A10				Compound 2B2				Compound 2A10	
	Germination (%)									
Treatment (μM)	*Ub*	*Ud*	*Bp*	*Ls*	*Ub*	*Ud*	*Bp*	*Ls*	Treatment (μM)	*Bp*
0	31	54	70 ab **	51	31	54	70 a	48	0	70 ab **
400	37	45	0c *	32	33	60	56 aB	57	50	75 aA **
600	29	33 *	0c *	35	32	40	28 bB *	50	100	56 bA **
800	24	27 * and **	0c *	32	30	37 *	28 bB *	50	200	55 bA **
400 Diuron	30	48	0c *	48	30	48	0c *	45	400 Diuron	0c *
Mean	30.5 ± 11	41.7 ± 10.0	64.2 ± 9.1	38 ± 12.3	31.5 ± 12.0	48 ± 10.4	45 ± 6.4	50 ± 13.7	Mean	
	Germination speed index (GSI)									
Treatment (μM)	*Ub*	*Ud*	*Bp*	*Ls*	*Ub*	*Ud*	*Bp*	*Ls*	Treatment (μM)	*Bp*
0	1.4	2.9	2.5 ab	1.8 ab	1.4	2.9	2.5 a	1.8b	0	2.5 ab
400	2.0	2.5	ND	1.4 bB **	1.8	3.5	1.7 a	3.9 aA *	50	2.2 a
600	1.7	2.1	ND	1.9 abB **	1.2	2.1	0.8 bB *	2.9 abA	100	1.5 bA
800	0.8	1.4 * and **	ND	1.2 bB **	1.0	2.2	0.7 bB *	2.5 bA	200	1.3 bA
400 Diuron	1.2	2.7	ND	2.9 a	1.2	2.7	ND *	2.9 ab	400 Diuron	ND
Mean	1.4 ± 0.4	2.3 ± 0.6	1.7 ± 0.3	1.8 ± 0.6	1.4 ± 0.4	2.7 ± 0.5	1.3 ± 0.3	2.7 ± 0.7	Mean	1.7 ± 0.3
	Germination rate of 50% of total seed population (T50; days)									
Treatment (μM)	*Ub*	*Ud*	*Bp*	*Ls*	*Ub*	*Ud*	*Bp*	*Ls*	Treatment (μM)	*Bp*
0	4.7	4.0	4.8c	5.7 a **	4.7	4.0	4.8	5.7 a **	0	4.8c
400	3.7	3.8	ND	3.7c *	3.8	4.1	5.7	2.9c *	50	6.6b *
600	4.5	4.2	ND	3.9c *	4.3	3.1	6.5	3.3c *	100	7.1 ab *
800	4.7	4.0	ND	4.8b **	4.1	3.5	7.1	4.3b *	200	8.2 a *
Diuron	4.4	3.6	ND	3.0 bc *	4.4	3.6	ND	3.3c *	400 Diuron	ND
Mean	4.4 ± 0.9	3.9 ± 1.4	6.7 ± 0.6	4.2 ± 0.5	4.3 ± 0.9	3.7 ± 1.1	6.0 ± 0.9	3.8 ± 0.6	Mean	6.7 ± 0.6

The values are the means of experiments performed with five replicates. The different lowercase letters indicate significant differences among the treatments (within a column) for each plant species according to Tukey's test ($P < 0.05$). The single asterisks (*) indicate significant differences between a treatment and the negative control (0 μM), while the double asterisks (**) indicate significant differences between a treatment and the positive control (400 μM diuron) according to Dunnett's test ($P < 0.05$). ND, not determined because the treatment was lethal to the seeds.

Table 2
Effects of compounds 2A10 and 2B2 or by diuron (control) on the development of roots and leaves of *Urochloa brizantha* (*Ub*), *Urochloa decumbens* (*Ud*), *Bidens pilosa* (*Bp*), and *Lactuca sativa* (*Ls*).

	Compound 2A10			Compound 2B2				Compound 2A10	
	Root length (mm)								
Treatment (μM)	*Ub*	*Ud*	*Ls*	*Ub*	*Ud*	*Bp*	*Ls*	Treatment (μM)	*Bp*
0	40.6 a **	50.8 a **	23.6 a **	40.6c **	50.8b **	50.0 a **	23.6 a **	0	50.0 a **
400	30.8 bB * and **	46.6 aB **	23.1 a **	69.8 aA * and **	67.3 aA * and **	29.3 bB **	26.8 a **	50	52.9 aA **
600	29.5b * and **	26.8 bB * and **	21.8 a **	27.8 d * and **	40.3 bA **	28.8 bA **	21.3 a **	100	7.3 cB * and **
800	18.0 cB * and **	31.5b * and **	24.2 a **	53.4 bA * and **	26.6c * and **	19.8 cB **	29.8 a **	200	32.0 bA * and **
400 Diuron	1.0 d *	1.0c *	13.6b *	1.0 e *	1.0 d *	1.0 d	13.6b *	400 Diuron or 400 2A10	ND
Mean	23.8 ± 1.9	31.2 ± 6.3	21.3 ± 2.6	38.3 ± 3.4	37.0 ± 5.3	32.0 ± 3.9	23.0 ± 3.2	Mean	35.6 ± 3.9
	Leaf area (mm^2)								
Treatment (μM)	*Ub*	*Ud*	*Ls*	*Ub*	*Ud*	*Bp*	*Ls*	Treatment (μM)	*Bp*
0	90.4 a **	39.5 ab	12.9	90.4 a **	39.5b	7.0 a **	12.9c	0	7.0 a
400	61.3b * and **	42.8 a **	12.6B	60.4b * and **	45.8 a * and **	5.0 bB **	20.9 abA * and **	50	7.3 aA
600	62.2b * and **	31.8 cB *	12.5B	60.2b * and **	46.5 aA * and **	5.4 abA **	21.8 aA * and **	100	4.3 bB *
800	40.8 cB * and **	39.8 abA	12.2B	99.5 aA **	33.1 cB *	6.7 a **	18.9 bA * and **	200	7.1 a
400 Diuron	22.8 d *	34.1 bc	10.7	22.8c *	34.1 bc	0c	10.7c	400 Diuron or 400 2A10	ND
Mean	55.5 ± 6.9	37.6 ± 3.2	12.2 ± 1.1	66.7 ± 7.3	39.8 ± 2.8	6.0 ± 1.1	17 ± 1.5	Mean	6.4 ± 0.8

The different lowercase letters indicate significant differences among the treatments within a plant species (column), while the different uppercase letters indicate significant differences among plant species within a treatment (line) according to Tukey's test ($P < 0.05$). The single asterisks (*) indicate significant differences in relation to the negative control (0 μM), while the double asterisks (**) indicate significant differences in relation to the positive control (400 μM diuron) according to Dunnett's test ($P < 0.05$). Diuron (400 μM) was used as a reference for the herbicides. ND, not determined because the treatment was lethal to the seeds.

brizantha because it stopped the root growth and led to the development of leaves that were 75% smaller than those of the negative control seedlings (Table 2; $P < 0.0004$). The treatment of *U. decumbens* seeds with 2A10 (≥600 μM) and 2B2 (800 μM) inhibited seedling root growth by 42–48% and leaf expansion by 16–20% (Table 2; $P < 0.001$). In fact, these treatments were as effective as those with diuron with respect to the inhibition of leaf growth. A lower concentration of 2B2 (400 μM), however, led to the elongation of *U. decumbens* roots such that they were 32% longer than those of the negative control (Table 2; $P < 0.001$). In general, 800 μM 2A10, but not 800 μM 2B2, negatively impacted the development of *U. brizantha* seedlings ($P < 0.0004$). Likewise, at 600 μM, 2A10 was effective on *U. decumbens,* whereas its sulphur-analogue 2B2 at the same concentration had no effect ($P < 0.0001$).

Similar to the effects obtained with 400 μM diuron, concentrations equal to or greater than 400 μM 2A10 were lethal to *B. pilosa*, as demonstrated by a lack of seed germination (Table 1). Even at 100 μM, a concentration that caused a 60% decrease in seed germination (Table 1), 2A10 markedly repressed the root elongation of *B. pilosa* seedlings by 85% and impaired leaf expansion by 39% (Table 2; $P < 0.0001$). The root length of *B. pilosa* seedlings originating from seeds germinated in the presence of 400 and 600 μM 2B2 or in the presence of 800 μM 2B2 was 42% (on average) and 60% shorter than that of the negative control seedlings, respectively (Table 2; $P < 0.0001$). Compared with that of the negative control, the leaf area in the presence of 400 μM 2B2 decreased by only 29% (Table 2; $P < 0.001$). Moreover, compared to the positive control, neither 2A10 nor 2B2 at concentrations up to 800 μM compromised the growth of the non-weed species *L. sativa* (Table 2; $P > 0.05$). In contrast, the leaves of *L. sativa* seedlings originating from seeds treated with 2B2 were 60% larger than those of the control seedlings (Table 2; $P < 0.001$).

Overall, 800 μM 2A10 caused leaf chlorosis in *U. brizantha* seedlings (Fig. 2-F and F.1), and compared with the control, 600 μM 2B2 reduced the leaf pigmentation and blocked secondary root formation in *U. decumbens* (Fig. 2-H) (Fig. 2-B). In contrast, compared with the control, 400 μM diuron completely prevented the development of roots in both *Urochloa* species (Fig. 2-G and I) (Fig. 2-B and C). Pre-emergent treatment with 100 μM 2A10 yielded severely chlorotic *B. pilosa* seedlings (Fig. 2-D), whereas some seedlings exhibited a necrotic root tip after treatment with 800 μM 2B2 (Fig. 2-E), which was not observed in the control seedlings (Fig. 2-A).

Accumulation of chloroplast pigments in leaves in response to 2A10 and 2B2

U. brizantha seedlings treated with 2A10 or 2B2 (600 μM) presented chlorophyll *a*, chlorophyll *b* and carotenoid contents that were 34%, 45% and 35% lower than those in the negative control seedlings (Table 3; $P < 0.001$). Greater concentrations (800 μM) of 2A10 and 2B2 further reduced the levels of chlorophyll *a* by 50% ($P < 0.001$). Compared with the control, diuron (400 μM) reduced the accumulation of chlorophyll *a* and *b* and carotenoids in *U. brizantha* leaves by 82, 89 and 68%, respectively (Table 3; $P < 0.001$). Conversely, compared with those in the *U. decumbens* control seedlings, the levels of chlorophyll *a* in *U. decumbens* seedlings originating from seeds geminated in the presence of 2A10 (≥400 μM) were 31% lower. 2A10 at 400 and 600 μM reduced the amount of chlorophyll *b* in *U. decumbens* leaves by 50 and 30%, respectively (Table 3; $P < 0.0009$). 2B2 (at the tested concentrations) had no effect on the levels of chlorophylls *a* or *b* in the *U. decumbens* leaves ($P > 0.5$). Compared to those in the negative control, the levels of carotenoids in the leaves of this weed species treated with 2A10 at 600 μM and 800 μM were 49% and 74% lower, whereas a modest decrease of 10% in the leaves of seedlings treated with 600 μM 2B2 was recorded (Table 3; $P < 0.001$).

Notably, 2A10 (≥50 μM) effectively inhibited the accumulation of chlorophyll *a* and *b* and carotenoids in *B. pilosa* leaves by 50%, 45% and 66%, respectively. 2B2 affected the levels of chloroplast pigments in *B. pilosa* at concentrations of only 400 and 800 μM; at these concentrations, compared with the negative control, 2B2 reduced the production of chlorophyll *a* by 28% (on average) and chlorophyll *b* by 56% and 27%, respectively (Table 3; $P < 2 \times 10^{-16}$). Diuron at 400 μM and 2A10 (≥400 μM) were lethal to *B. pilosa.* The chlorophyll *a* content in *L. sativa* leaves was affected by 2A10 and 2B2 only at the highest concentration tested (800 μM) and by 400 μM diuron, which resulted in a chlorophyll *a* content

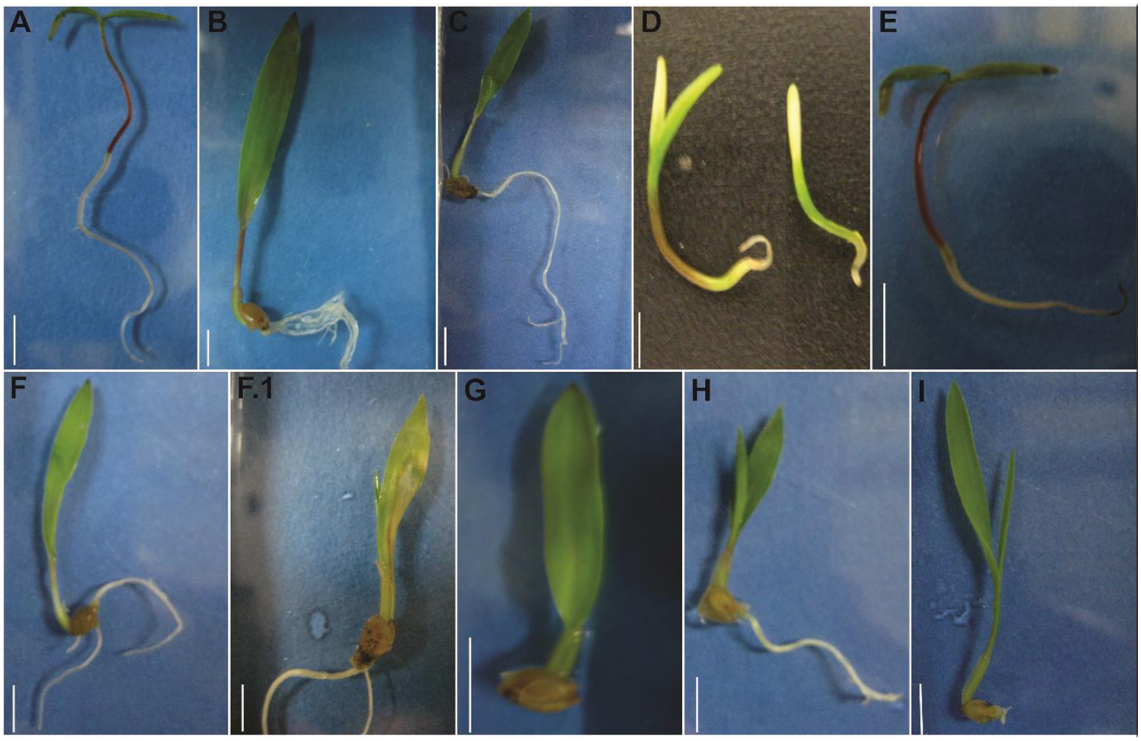

Fig. 2. Examples of developmental anomalies caused by 2A10, 2B2 and diuron in seedlings of select weed species. *B. pilosa* seedlings (10 days old) originating from seeds germinated in the absence (**A**) or presence of 100 μM 2A10 (**D**) or 800 μM 2B2 (**E**); *U. brizantha* seedlings (15 days old) originating from seeds germinated in the absence (**B**) or presence of 800 μM 2A10 (**F**), which show leaf chlorosis (**F.1** inset), or 400 μM diuron (**G**); *U. decumbens* seedlings (15 days old) originating from seeds germinated in the absence (**C**) or presence of 600 μM 2B2, which show lack of secondary root formation (**H**), or 400 μM diuron (**I**). The images are representative of three independent experiments. Vertical bars = 0.5 cm.

that was 33% lower than that in the control seedlings. Compared with the control, both 2A10 and 2B2 (≥400 μM) reduced the level of chlorophyll *b* in *L. sativa* by 62.5%; in addition, 2A10 (400 μM) stimulated the accumulation of carotenoids by 24%, whereas 2B2 (600 μM and 800 μM) resulted in a 45% lower accumulation of carotenoids in *L. sativa* leaves. At the concentration tested, diuron did not affect the carotenoid contents in *L. sativa* (Table 3; $P > 0.9$).

Effects of 2A10 and 2B2 on biomass accumulation of whole seedlings

The biomass of *U. brizantha* and *U. decumbens* seedlings originating from seeds germinated in the presence of 800 μM 2A10 was approximately 2-fold greater than that of the negative control seedlings (Table 4; $P < 0.001$). When applied at lower concentrations, this compound had no effect on the biomass accumulation of the monocot weeds (Table 4; $P > 0.2$). Treatment with 600 μM 2B2 triggered a 41% increase in the biomass of *U. decumbens* seedlings (Table 4; $P < 0.001$), but even at greater concentrations, this compound did not greatly affect the dry matter of *U. brizantha*. Diuron (400 μM) induced the accumulation of biomass in *U. brizantha* (by 2.3-fold).

Diuron (400 μM) and 2A10 (≥400 μM) were lethal to *B. pilosa* seeds. Compared with the negative control, lower concentrations of 2A10 (50 μM and 200 μM) increased the accumulation of biomass in *B. pilosa* by 26% (on average), while 2B2 (400 μM) reduced the biomass by 14% (Table 4; $P < 0.001$). Compared to that in the *L. sativa* negative control seedlings, the accumulation of biomass in the *L. sativa* seedlings treated with 2A10 (400 μM and 600 μM), 2B2 (600 μM) and diuron decreased by 13%, 22% and 42%, respectively (Table 4; $P < 0.001$).

Discussion

Weed species are detrimental to crop production, and 40% of the known invasive species worldwide (approximately 250) belong to the Poaceae and Asteraceae families (www.embrapa.br/en/tema-plantas-daninhas/sobre-o-tema). The invasive species *U. brizantha* and *U. decumbens* (Poaceae; monocot) as well as *B. pilosa* (Asteraceae; dicot) were selected to investigate the potential of (thio)urea derivatives as pre-emergence herbicides. *L. sativa* (Asteraceae; dicot) was used as a non-weed model to test the selectivity of the compounds towards the studied weed species [31,32], and diuron (a urea derivative) was used as a pre-emergence herbicide Ref. [33].

The urea derivative 2A10, but not the thio-derivative 2B2, was lethal to *B. pilosa* seeds at concentrations greater than 200 μM, and this effect was comparable to that of diuron, a well-known urea-derived herbicide [33]. Even at concentrations lower than 200 μM, 2A10 negatively affected the germination rate of *B. pilosa* seeds, the degree of leaf expansion and the accumulation of chlorophylls and carotenoids in the leaves, and this treatment resulted in the development of few roots. The disruption of root growth is a typical effect of auxin-like herbicides such as 2,4-dichlorophenoxyacetic acid (2,4D) [34] and is not a common effect of substituted urea herbicides. Although not tested, it is possible that 2A10 stimulated the flow of auxin towards the roots, where the accumulation of this plant hormone would inhibit organ development [35]. The lethal effects of diuron on *B. pilosa* corroborates those reported elsewhere [36].

Although less efficient than its *oxo*-analogue, 2B2 reduced the germination ability of *B. pilosa* seeds and considerably slowed the

Table 3
Accumulation of chloroplast pigments in *Urochloa brizantha*, *Urochloa decumbens*, *Bidens pilosa* and *Lactuca sativa* leaves after treatment with 2A10 or 2B2.

	Urochloa brizantha					
	Chlorophyll *a* (mg g^{-1} FW)		Chlorophyll *b* (mg g^{-1} FW)		Carotenoids (mg g^{-1} FW)	
Treatment (μM)	2A10	2B2	2A10	2B2	2A10	2B2
0	68.2 a **	68.2 a **	23.0 a **	23.0 a **	9.4 a **	9.4 a **
400	63.6 a **	62.6 a **	21.2 a **	19.7 a **	8.2 a **	8.9 a **
600	45.0b * and **	45.1b * and **	13.9b * and **	14.1b * and **	6.1b * and **	6.7b * and **
800	31.8c * and **	36.0c * and **	11.6b * and **	11.3b * and **	5.8b * and **	5.7b * and **
400 Diuron	12.6 d *	12.6 d *	2.8c *	2.8c *	3.1c *	3.1c *
Mean	44.2 ± 4.3	44.9 ± 4.8	14.5 ± 1.8	14.1 ± 1.7	6.5 ± 1.0	6.8 ± 0.8
	Urochloa decumbens					
	Chlorophyll *a* (mg g^{-1} FW)		Chlorophyll *b* (mg g^{-1} FW)		Carotenoids (mg g^{-1} FW)	
Treatment (μM)	2A10	2B2	2A10	2B2	2A10	2B2
0	85.9 a **	85.9 a **	16.1b **	16.1b **	9.3 a **	9.3 ab **
400	52.2 bB ** and **	88.5 aA **	7.2 dB *	24.0 aA * and **	9.6 a **	10.6 a **
600	61.1 bB * and **	79.7 aA **	11.6 cB * and **	21.0 aA * and **	4.7b *	8.4b **
800	63.9 bB * and **	88.7 aA **	31.1 aA * and **	23.3 aB * and **	2.4c *	9.4 ab **
400 Diuron	28.6c *	28.6b *	6.4 d *	6.4c *	3.4 bc *	3.4c *
Mean	58.3 ± 7.7	74.3 ± 7.2	14.5 ± 1.9	18.2 ± 1.3	5.9 ± 1.0	8.2 ± 1.0
	Bidens pilosa					
	Chlorophyll *a* (mg g^{-1} FW)		Chlorophyll *b* (mg g^{-1} FW)		Carotenoids (mg g^{-1} FW)	
Treatment (μM)	2A10	2B2	2A10	2B2	2A10	2B2
0	99.2 a	99.2 a	33.3 a	33.3 a	19.4 a	19.4 a
50 (2A10) or 400 (2B2)	47.5 bB *	69.4 bA	18.4b *	14.7c *	8.8 bB *	22.2 aA
100 (2A10) or 600 (2B2)	48.8 bB *	95.2 aA	18.2 bB *	30.4 aA	8.9 bB *	22.9 aA
200 (2A10) or 800 (2B2)	51.7 bB *	71.9 bA	18.1 bB *	24.4 bA *	10.8b *	18.4 a
400 Diuron or 400 2A10	ND	ND	ND	ND	ND	ND
Mean	61.8 ± 3.7	83.9 ± 7.0	22.0 ± 1.4	25.7 ± 4.5	12 ± 0.9	20.7 ± 2.3
	Lactuca sativa					
	Chlorophyll *a* (mg g^{-1} FW)		Chlorophyll *b* (mg g^{-1} FW)		Carotenoids (mg g^{-1} FW)	
Treatment (μM)	2A10	2B2	2A10	2B2	2A10	2B2
0	21.5c **	21.5 a **	10.2 a	10.2 a	5.7 bc	5.7 a
400	27.9 bA **	20.2 aB **	3.0b * and **	4.7b * and **	6.8 abA	2.9 bB * and **
600	33.4 aA * and **	19.3 aB **	3.6b * and **	3.0b * and **	7.8 aA **	4.0 abB
800	15.9 d	13.6b *	5.1b * and **	3.5b * and **	4.6 cA	3.3 bB * and **
400 Diuron	12.7 d *	12.7b *	10.1 a	10.1 a	5.3 bc	5.3 a
Mean	22.3 ± 3.2	17.5 ± 2.0	6.4 ± 1.7	6.3 ± 1.8	6.1 ± 1.1	4.3 ± 0.9

The different lowercase letters indicate significant differences among the treatments within a plant species (column), while the different uppercase letters indicate significant differences among plant species within a treatment (line) according to Tukey's test ($P < 0.05$). The single asterisks (*) indicate significant differences in relation to the negative control (0 μM), while the double asterisks (**) indicate significant differences in relation to the positive control (400 μM diuron) according to Dunnett's test ($P < 0.05$). ND, not determined because the treatment was lethal to the seeds.

Table 4
Biomass accumulation in seedlings of *Urochloa brizantha* (*Ub*), *Urochloa decumbens* (*Ud*), *Bidens pilosa* (*Bp*), and *Lactuca sativa* (*Ls*) after treatment with 2A10 or 2B2.

	Biomass (% DW)								
	Compound 2A10			Compound 2B2				Compound 2A10	
Treatment (μM)	*Ub*	*Ud*	*Ls*	*Ub*	*Ud*	*Bp*	*Ls*	Treatment (μM)	*Bp*
0	10.7b **	10.2b	5.9 ab **	10.7b **	10.2b **	7.1 ab **	5.9 a **	0	7.0b
400	13.7 bA **	13.5b	5.0b **	9.4 bB **	11.1 ab	6.1 bB **	5.3 ab **	50	9.0 aA *
600	14.2 bA **	12.4b	5.3b **	10.5 bB **	14.5 a *	6.9 abA **	4.6 bc *	100	ND
800	22.2 aA *	22.0 aA * and **	6.9 aA **	9.4 bB **	12.3 abB *	7.8 a **	5.1 abB **	200	8.6 a *
400 Diuron	25.5 a *	12.6b	3.5c *	25.5 a *	12.6 ab *	0c *	3.5c *	400 Diuron or 400 2A10	ND
Mean	17.3 ± 2.7	14.1 ± 2.2	5.3 ± 0.7	13.1 ± 1.6	12.1 ± 1.1	7.0 ± 0.8	4.9 ± 0.6	Mean	6.2 ± 0.6

Seeds were treated with 2A10 or 2B2 at the indicated concentrations, and 15-day-old (*U. brizantha* and *U. decumbens*) or 10-day-old (*B. pilosa* and *L. sativa*) seedlings were dried. The biomass was determined as the percentage of dry weight (DW) in relation to the fresh weight. The different lowercase letters indicate significant differences among the treatments within a plant species (column), while the different uppercase letters indicate significant differences among plant species within a treatment (line) according to Tukey's test ($P < 0.05$). The single asterisks (*) indicate significant differences in relation to the negative control (0 μM), while the double asterisks (**) indicate significant differences in relation to the positive control (400 μM diuron) according to Dunnett's test ($P < 0.05$). DW, dry weight. ND, not determined either because the weight of the dry matter was lower than the detection limit of the scale used (100 μM 2A10 treatment) or because the treatment (400 μM diuron) was lethal to the seedlings.

germination rate of the seeds that remained competent during the 10-day experimental period. The process of root development in the successfully germinated *B. pilosa* seedlings was severely inhibited by 2B2, likely due to the delay in the germination of the seeds [37] and dysfunctional cell respiration [38]. Remarkably, neither 2A10 nor 2B2 affected the germination of the non-weed species

L. sativa. The seedlings of *L. sativa* originating from seeds that geminated in the presence of 2A10 or 2B2 also exhibited normal development, as demonstrated by their normal root and shoot growth and biomass accumulation. Additionally, 2B2 stimulated the expansion of *L. sativa* leaves, although it resulted in a lower accumulation of chloroplast pigments. The decrease in the levels of chlorophyll *b* in *L. sativa* leaves in response to 400 μM or 600 μM 2A10 was compensated by an increase in carotenoid levels. In addition to the antioxidant role of carotenoids, both types of pigments play important roles in the light-harvesting complex of thylakoid membranes during photosynthesis. Other organic substances reportedly compromise the growth and development of *B. pilosa*, but none are structurally related to 2A10 or 2B2. Eugenol, a natural phenolic compound, was shown to reduce the germination of *B. pilosa* seeds by 61% at a concentration 2.5-fold greater (1 mM) than that of 2A10 necessary to abolish *B. pilosa* germination. In contrast, treatment with 50 μM or 1 mM eugenol resulted in seedling roots that were 11% and 69% shorter than those of the control, respectively, whereas 1 mM concentrations of this phenolic agent compromised the production of chlorophyll by 40% [39]. Although 32 mg L^{-1} berberine (equivalent to 95 μM), a benzylisoquinoline alkaloid, had no effect on the germination of *B. pilosa* seeds, 14 days of treatment with this compound resulted in an 86% loss in seedling fresh weight and inhibited the growth of the primary roots by 95% [40]. An assessment of four synthetic tetraoxanes (at 1 mM) for their potential use as post-emergence herbicides revealed that the compounds inhibited the biomass accumulation in the roots and shoots of *B. pilosa* by 47–90% and 32–89%, respectively [41]. These compounds, however, were highly phytotoxic to the roots and shoots of the non-weed species *Cucumis sativus* and *Sorghum bicolor* [41].

The compounds 2A10, 2B2 and even diuron failed to function as pre-emergence herbicides against *U. brizantha*, but *U. brizantha* seedlings originating from seeds treated with these compounds presented shorter roots (2A10) or arrested root growth (diuron), less expanded leaves (2A10 or diuron) and reduced contents of chloroplast pigments (2A10 and 2B2). The post-emergent use of three synthetic tetraoxanes (1 mM) inhibited the growth of *U. brizantha* roots and shoots by at least 95% [41]. Both 2A10 and 2B2 effectively inhibited the germination of *U. decumbens* seeds and delayed root protrusion without impacting root and leaf growth, and these effects were obtained with all tested concentrations of these compounds, except for 800 μM 2B2, which reduced the root growth. 2A10 reduced the accumulation of chlorophylls and carotenoids without notably affecting the external features of seedlings, and 2B2 blocked the formation of lateral roots in *U. decumbens* seedlings. The overall effects of 2B2 on *U. decumbens* were more striking than those of 2A10 because a lack of lateral roots makes it relatively more difficult for plants to access soil nutrients. These results suggest that 2A10, 2B2 and diuron can potentially act as post-emergence herbicides against *U. brizantha* and/or *U. decumbens*. All the compounds tested affected the accumulation of at least one type of chloroplast pigment, which would expectedly result in a lower accumulation of biomass due to changes in net photosynthesis [42]. However, the accumulation of biomass by itself is not enough to predict the competitiveness of a weed species in relation to non-weed species [8]. This finding is attested by the commercial post-emergence herbicides dicamba and glyphosate also stimulating the accumulation of biomass (20–40%) in the roots of the weed *Liriope spicata* at 90 days after treatment [43].

The lethal effect of 2A10 (but not 2B2) on *B. pilosa* seeds is likely due to the presence of an oxygen atom in the urea moiety, as 2B2 bears a sulphur atom instead of this oxygen atom. This supposition is also supported by diuron having an oxygen atom in the urea moiety and the same effectiveness of diuron as 2A10 against *B. pilosa* seeds.

Conclusions

In conclusion, the pre-emergent use of the urea derivative 2A10 and the thiourea derivative 2B2 compromises the seed germination and development of the weed species *U. brizantha*, *U. decumbens* and *B. pilosa* to different extents. Both compounds were selective to the investigated weed species because *L. sativa*, a species that is widely used in phytotoxic tests, exhibits normal development even in the presence of relatively high concentrations of 2A10 or 2B2. 2A10 was also efficient against *U. brizantha*, whereas the effects of 2B2 were more prominent in *U. decumbens*. Overall, 2A10 was shown to be an efficient pre-emergence herbicide against the dicot *B. pilosa*, while 2B2 seemed to be a promising post-emergence herbicide against *U. decumbens*. Therefore, 2A10 and 2B2 are interesting choices for subsequent investigation of the mechanism of action and interaction with soil components as well as leading choices for the design of new phytotoxic compounds for the control of the investigated weed species.

Conflict of interest

The authors declare no conflict of interest.

Compliance with Ethics Requirements

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

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