Seed bank and control of *Rottboellia exaltata* using clomazone alone and in combination with other herbicides

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**ABSTRACT**

The objective was to study the seed bank and control of *Rottboellia exaltata* L.f. in sugarcane areas with low and high infestation levels using clomazone alone and in combination with other herbicides. Two experiments were conducted using commercial sugarcane production areas. In both areas, the sugarcane was mechanically harvested without burning, with straw ground cover at 10 and 9 tons ha⁻¹ for the first (high infestation) and second (low infestation) experiments, respectively. Treatments were clomazone alone and in combination with tebuthiuron, hexazinone, imazapyr, flumioxazin, amicarbazone or isoxaflutole, besides sulfometuron methyl + diuron + hexazinone, and two controls untreated with herbicide: a weedy control and a hand-weeded control. In the second experiment, the clomazone + imazapyr treatment was replaced with a clomazone + ametryn treatment. Under the conditions of low (6.1 plants m⁻²) *R. exaltata* infestation, there was no difference among the herbicide treatments. With the increased infestation (19.4 plants m⁻²), the clomazone + imazapyr, clomazone + isoxaflutole, and sulfometuron + diuron + hexazinone treatments differed from the others and promoted better control, as reflected in the soil seed bank of the weed species and in the sugarcane stalk yield.

**Key words:** application of preemergence herbicides, itchgrass, raw sugarcane

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**Banco de sementes e controle de Rottboellia exaltata com clomazone isolado e em associação com outros herbicidas**

RESUMO

Objetivou-se, com este trabalho, estudar o banco de sementes e o controle de *Rottboellia exaltata* L.f. com clomazone, isolado e em associação com outros herbicidas, em áreas de cana-soca com baixa e alta infestação. Dois experimentos foram desenvolvidos em áreas de produção comercial de cana-de-açúcar. Nas duas áreas a cana foi colhida mecanicamente sem queima, com a manutenção de 10 e 9 t ha⁻¹ de palha sobre o solo, respectivamente para primeiro (alta infestação) e segundo (baixa infestação) experimentos. Os tratamentos estudados foram clomazone isolado e em associação com o tebuthiuron, hexazinone, imazapyr, flumioxazin, amicarbazone ou isoxaflutole, além de sulfometuron-methyl + diuron + hexazinone e duas testemunhas sem aplicação. No segundo experimento o tratamento clomazone + imazapyr foi substituído por clomazone + ametryn. Em condições de baixa (6.1 plantas m⁻²) infestação de *R. exaltata*, não houve diferença entre os tratamentos de herbicidas estudados. Com o aumento da infestação (19.4 plantas m⁻²), os tratamentos clomazone + imazapyr, clomazone + isoxaflutole e sulfometuron + diuron + hexazinone diferiram dos demais e promoveram melhor controle, com reflexos no banco de sementes dessa espécie, no solo e na produção de colmos de cana.

**Palavras-chave:** aplicação de herbicidas em pré-emergência, capim-camalote, cana crua
Introduction

*Rottboellia exaltata* L.f., synonym *R. cochinchinensis* (Lour.) Clayton, is an annual or perennial plant depending on environmental conditions and reproduces by seeds or from pieces of stem that have nodes with buds (Kissmann, 1997). Apart from the losses caused by competition for water, light, nutrients and space, decaying plant debris of this species releases phytotoxic compounds into the soil that can inhibit the germination and growth of adjacent species, whether cultivated plants or weeds (Kobayashi et al., 2008; Meksawat & Pomprom, 2010). *R. exaltata* is the primary weed in at least 18 crops in Africa, Asia, Central and South America, the United States, Australia and Papua New Guinea (Holm et al., 1991; Kissmann, 1997; Anning & Yeboah-Gyan, 2007). In Brazilian sugarcane plantations, *R. exaltata* occurs fairly frequently in Rio de Janeiro (Oliveira & Freitas, 2008), São Paulo, Paraná, and Mato Grosso do Sul, and there are reports of its occurrence in the northern region and other states in the Midwest of Brazil (Kissmann, 1997).

There is little information in the literature concerning the chemical control of *R. exaltata* in sugarcane, especially for herbicides applied preemergence. It is known, however, that this type of management is costly because as many as six herbicide applications may be required during the crop cycle (Oliveira & Freitas, 2009).

In this regard, it was reported that the herbicide combinations trifloxysulfuron-sodium + ametryn (0.037 + 1.465 kg ha⁻¹, respectively), sodium hydrogen methylarsonate (MSMA) + diuron (2.88 + 1.12 kg ha⁻¹), and diuron + paraquat (0.30 + 0.60 kg ha⁻¹) were effective in controlling *R. exaltata* when sprayed on plants having six to eight leaves (Freitas et al., 2004). The herbicides clomazone (1.20 kg ha⁻¹) and imazapyr (0.20 kg ha⁻¹) were effective in the preemergence control of this species, regardless of the level of straw (5, 10, and 15 tons ha⁻¹) on the soil surface (Correia et al., 2013). Flumioxazin (0.25 kg ha⁻¹) was also effective but only on bare soil. Even with 60 mm of rainfall accumulated over four days after application, the amount of flumioxazin leached into the soil was insufficient to ensure the same control observed with application on bare soil (Correia et al., 2013).

Furthermore, the dynamics of *R. exaltata* infestation may be affected by mechanized harvesting without sugarcane burning and also by the maintenance of straw on the soil surface. With this harvesting system, drastic reductions in the amount of flumioxazin leached into the soil would require longer residual control in this condition.

Materials and Methods

Two experiments were conducted in commercial sugarcane production areas from September 2011 to July 2012.

The first experiment was performed at Campestre Farm in the municipality of Igarapava, São Paulo State, SP, in a third roatton harvest sugarcane variety RB855453; the second experiment was installed at Água Branca Farm in the municipality of Barrinha, SP, in a fourth roatton harves of the same variety of sugarcane. In the two areas, the cane crops were mechanically harvested without burning while maintaining 10 and 9 tons ha⁻¹ of straw cover over the soil surface for the first and second experiments, respectively.

The soil of the two experimental areas is clayey, and the climate of the region according to the Köppen classification is considered temperate humid with a dry winter and hot summer.

For both experiments, the experimental design was a randomized block with four replications. Eight herbicide treatments were studied (Tables 1 and 2) together with two controls untreated with herbicide: one weedy control and one hand-weeded control. In the second experiment, the clomazone + imazapyr treatment was replaced by treatment with clomazone + ametryn because of the possibility of greater imazapyr phytotoxicity to sugarcane with increased soil water availability. In addition, the doses of the herbicides used were higher in the first experiment than in the second. This change was made due to a scarcity of water at the time of product application, which would require longer residual control in the soil because canopy closure by sugarcane plants is slower under this condition.

Each plot was 6.0 m wide (4 rows of sugarcane) and 8.0 m in length, totaling 48.0 m².

Herbicides were applied at a spray volume of 200 L ha⁻¹, at constant pressure of 3.6 kgf cm⁻², with nozzles spaced in 0.5 m, by using a CO₂ pressurized backpack sprayer equipped with six flat-fan spray nozzle (TT 11002). In the first experiment, the herbicides were applied to dry soil on 09/24/2011 from 3:15 p.m. to 4:17 p.m. at air temperature of 30.0 to 31.1°C, soil temperature of 27.0 to 27.3°C, air relative humidity of 34 to 35%, wind speed of 0 to 1.6 km h⁻¹, and cloud cover of 40 to 80%. In the second experiment, herbicides were applied to moist soil on 10/15/2011 from 9:30 a.m. to 11:00 a.m. at air temperature of 30.1 to 32.6°C, soil temperature of 24.2 to 25.5°C, air relative humidity of 66 to 71%, wind speed of 1.3 to 6.3 km h⁻¹, and cloud cover of 70-100%.

At 33, 68, 111, and 140 days after the application (DAA) of the herbicides in the first experiment and at 35, 59, 90, and 120 DAA in the second experiment, control levels of *R. exaltata* were visually evaluated and assigned scores ranging from 0 to 100%, where zero represents absence of symptoms in the weed and 100% represents death of the weed. Possible damage to sugarcane plants was also visually evaluated at 33, 68, and 111 DAA for the first experiment and at 13, 35, and 59 DAA for the second experiment using a scale from 0 to 100%, where zero represents no visual damage to the sugarcane plant and 100%
represents death of the plant. Sugarcane yield was quantified 249 and 265 DAA for the first and second experiment, respectively, by counting the number of cane stalks within a 12.0-m² area of each plot and collecting 20 stalks in a linear sequence. Based on the total number of stalks per 12.0-m² area and the weight of the 20 collected stalks, the sugarcane yield was estimated in tons per hectare.

In the first experiment, after stalk collection, composite soil samples (six subsamples per plot) were collected at a depth of 0-10 cm using a tube auger of 5-cm inner diameter. The soil samples were sent to the Weeds Laboratory at the Department of Crop Protection of FCAV/UNESP, Jaboticabal, SP, for the evaluation of the *R. exaltata* seed bank. At the laboratory, the samples were air dried and sieved (70-cm diameter x 4-cm rim) through a 0.2-mm stainless steel mesh to partially remove the soil solid fraction to allow seed sorting and counting.

For each plot, the data on the collected seeds were converted to the number of seeds per m² using a correction factor. This parameter was calculated by dividing the area of 10,000 cm², which is equivalent to 1.0 m², by the number of samples/m²/plot and the area of the auger. This value was then multiplied by the number of seeds detected per sample within the plot.

After sorting and counting, the seeds from each soil sample were sown in plastic pots with a 4-liter soil capacity. The substrate used was a mixture of soil, sand, and organic compost at a ratio of 3:1:1, respectively. Each pot represented the seeds quantified from each plot of the field experiment. At 28 days after sowing, the numbers of emerged plants as well as shoot dry matter were determined. Statistical significance was tested using the analysis of variance (ANOVA) F-test. When significant, treatment means were compared by Tukey’s test at the 5% probability level. The seed bank data were not statistically analyzed but were instead examined by comparing treatment means using frequency and graphical analyses.

Table 1. Percentage of *Rottboellia exaltata* control at 33, 68, 111, and 140 days after application (DAA) of herbicides on the sugarcane crop and in the weedy control untreated with herbicide (1st experiment, herbicide applied on Sept. 24th, 2011)

| Herbicide treatment | Dose (g ha⁻¹) | Control (%) by DAA |
|---------------------|---------------|-------------------|
|                     |               | 33    | 68       | 111   | 140   |
| 1-Clomazone         | 1600          | 77.50 a(1) | 63.75 b | 30.00 d | 30.00 d |
| 2-Clomazone +       | 1200          | 82.50 de | 77.50 ab | 46.25 cd | 46.25 cd |
| isoxaflutole        | 750           | 90.62 abc | 78.75 a | 58.75 abc | 58.75 abc |
| hexazinone          | 1200          | 94.38 a | 88.12 a | 78.75 ab | 78.75 ab |
| 3-Clomazone +       | 1200          | 81.88 de | 75.62 ab | 53.75 bcd | 53.75 bcd |
| imazapyr            | 325           | 83.75 bcd | 78.12 ab | 55.00 bcd | 55.00 bcd |
| 4-Clomazone +       | 1200          | 93.12 ab | 87.50 a | 81.25 a | 81.25 a |
| flumioxazin         | 200           | 90.00 abc | 85.00 a | 75.00 ab | 75.00 ab |
| hexazinone          | 1200          | -   | 0.00 | 0.00 | 0.00 |
| 5-Clomazone +       | 1200          | 8.72** | 6.45** | 10.28** | 10.28** |
| imazapyr            | 250           | 4.77 | 7.86 | 18.41 | 18.41 |
| diuron +            | 1200          | 9.80 | 14.78 | 26.13 | 26.13 |
| hexazinone          | 1050          | -   | 0.00 | 0.00 | 0.00 |
| 6-Clomazone +       | 1200          | 90.00 abc | 85.00 a | 75.00 ab | 75.00 ab |
| amicarbazone        | 225           | -   | 0.00 | 0.00 | 0.00 |
| 7-Clomazone +       | 1200          | 8.72** | 6.45** | 10.28** | 10.28** |
| isoxaflutole        | 425           | 4.77 | 7.86 | 18.41 | 18.41 |
| hexazinone          | 1507.5        | 9.80 | 14.78 | 26.13 | 26.13 |
| 8-Sulfometuron +    | 30.25         | -   | 0.00 | 0.00 | 0.00 |
| diuron +            | 425           | 90.00 abc | 85.00 a | 75.00 ab | 75.00 ab |
| hexazinone          | -             | -   | 0.00 | 0.00 | 0.00 |
| 9-Weedy control(2)  |               | -   | 0.00 | 0.00 | 0.00 |
| F                   |               | 8.72** | 6.45** | 10.28** | 10.28** |
| CV(1) (%)           |               | 4.77 | 7.86 | 18.41 | 18.41 |
| LSD(2)              |               | 9.80 | 14.78 | 26.13 | 26.13 |

** Significant at the 1% probability level using the ANOVA F-test.

(1) Means within columns followed by the same letter do not differ significantly by Tukey’s test at the 5% probability level. (2) The weedy control was not included in the statistical analysis. (3) Coefficient of variability. (4) Least significant difference.

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the soil seed banks. The population density of *R. exaltata* in the weedy controls was 19.4 and 6.1 plants m⁻² for the first and second experiments, respectively. These counts were performed at the time of the second control evaluation for each experiment. Thus, the presence of 9 and 10 tons ha⁻¹ of straw on the soil surface was not effective for weed management. Correia et al. (2013) reported that despite a reduction in *R. exaltata* seedling emergence with an increased amount of straw cover on the soil, the reduction was insufficient to achieve adequate levels of weed control, especially when the seeds were deposited near the soil surface.

In addition to its possible effects on the germination and emergence of weeds, straw originating from mechanized harvesting without sugarcane burning can also compromise the ability of residual herbicides to reach the soil. Depending on the physical and chemical characteristics of herbicides, such as solubility, vapor pressure and polarity, straw cover can have a greater or lesser influence on herbicidal effectiveness (Rodrigues, 1993). The timing and amount of rainfall or irrigation after herbicide application as well as changes in the chemical composition of decaying plant materials can greatly influence herbicide retention by straw cover (Correia et al., 2007). The herbicide on the straw that is not transferred into the soil is subject to losses from photodegradation, volatilization, and adsorption onto plant litter whose degree of decomposition or age can affect its ability to adsorb herbicides (Mersie et al., 2006).

In the first and second experiments, rainfall during the first fifteen days after the herbicide application was 15.9 and 88.86 mm, respectively. In the first experiment, the first rainfall (15.9 mm) occurred on the eighth day after application, whereas in the second experiment, rainfall (44.94 mm) occurred on the day of application (Figure 2).

According to Negrisoli et al. (2011), for the herbicide combination of clomazone + hexazinone (880 + 220 g ha⁻¹), only 2.5 mm of rainfall promoted herbicide leaching from sugarcane straw (5 tons ha⁻¹) into the soil.

In the second experiment, the amount and frequency of rainfall after herbicide application were sufficient to leach clomazone from the straw into the soil. It is believed based on the work of Carbonari et al. (2010) that leaching would have occurred in the first experiment because the accumulated volume of rainfall was 15.9 mm in 15 days and 111.5 mm in 22 days. In that study, the combination of clomazone and hexazinone was effective for the control of *Brachiaria decumbens* when applied over or under sugarcane straw cover or without straw cover regardless of the period without rain (0, 3, 7, 15, 30, and 60 days) (Carbonari et al., 2010). However, there was a trend toward reduced control levels for periods over 60 days without rain, mainly over the straw cover and soil, indicating that degradation occurs when the herbicide is subjected to extended periods of exposure on the surface of straws or soil without rainfall, which would enable its distribution throughout the soil profile.

Under potted conditions (greenhouse), Correia et al. (2013) obtained excellent control of *R. exaltata* by clomazone (1200 g ha⁻¹) with or without cane straw cover on the soil. By contrast, the application of this herbicide alone at 1600 g ha⁻¹
in the present study did not have the same efficacy. This result can be explained by the adequate soil moisture conditions in greenhouse compared with field experiments. In the above greenhouse experiment on the day of herbicide application, a rainfall depth equivalent to 25 mm was simulated, and when necessary, moisture was controlled daily by replenishing water in containers placed under the pots (Correia et al., 2013). Furthermore, the herbicide effects were evaluated only for 35 days in the greenhouse, whereas in the fields, the effects were evaluated until 140 DAA.

Regarding the sugarcane characteristics evaluated in the first experiment, the herbicide treatments significantly affected

| Herbicide treatment | Dose (g ha⁻¹) | Control (%) by DAA | 35     | 59     | 90     | 120    |
|---------------------|---------------|--------------------|--------|--------|--------|--------|
| 1-Clomazone         | 1200          | 90.62 a           | 82.50  | 75.62  | 75.62  |
| 2-Clomazone + tebuthiuron | 1000    | 93.75 a           | 90.62  | 84.38  | 84.38  |
| 3-Clomazone + hexazinone | 1000    | 93.12 a           | 86.25  | 83.12  | 83.12  |
| 4-Clomazone + ametryn | 1000    | 95.00 a           | 93.12  | 88.12  | 88.12  |
| 5-Clomazone + flumioxazin | 1000    | 85.00 a           | 86.25  | 81.25  | 81.25  |
| 6-Clomazone + amicarbazone | 1000   | 96.25 a           | 95.62  | 93.12  | 93.12  |
| 7-Clomazone + isoxaflutole | 1000    | 86.88 a           | 84.38  | 76.88  | 76.88  |
| 8-Sulfometuron + diuron + hexazinone | 29       | 91.88 a           | 91.88  | 86.25  | 86.25  |
| 9-Weedy control     | -             | 00.00             | 00.00  | 00.00  | 00.00  |

| CV (%)             | 09.71         | 09.48             | 11.73  | 11.73  |        |
| LSD (4)            | 21.10         | 19.97             | 23.26  | 23.26  |        |

(4) Not significant at the 5% probability level using the ANOVA F-test.

(1) Means within columns followed by the same letter do not differ significantly by Tukey’s test at the 5% probability level. (2) The weedy control was not included in the statistical analysis. (3) Coefficient of variability. (4) Least significant difference.

Figure 2. Daily rainfall values recorded from 09/20 to 10/31/2011 for the first experiment and from 10/10 to 11/20/2011 for the second experiment. Data derive from agroclimatic stations located 5.0 km from the experimental area.

Table 2. Percentage of *Rottboellia exaltata* control at 35, 59, 90, and 120 days after application (DAA) of herbicides on the sugarcane crop and in the weedy control untreated with herbicide (2nd experiment, herbicide applied on Oct. 15th, 2011).
the phytotoxicity score at 33 and 68 DAA as well as the number and yield of stalks (Table 3).

Regarding the phytotoxicity effects evaluated at 33 and 68 DAA, the clomazone + imazapyr combination resulted in major visual damage to the sugarcane plants, unlike the other treatments. However, by 111 DAA, the plants sprayed with this mixture had recovered and did not show any visual symptoms of phytotoxicity.

In the weedy control plots, there was a decreased number and yield of stalks, which differed significantly from the hand-weeded controls. Damage caused by _R. exaltata_ to the sugarcane plants resulted in significant losses in the crop yield capacity. Comparing the two experimental controls, there was a reduction of 56% in the sugarcane stalk yield in plants maintained throughout their life cycle with weeds.

The herbicide treatment plots did not differ from the hand-weeded control stalk number and yield. Herbicides did not compromise growth and development of sugarcane, either through their toxic effects on the cultivated plants or through their unsatisfactory weed control. In these types of studies, crop yield reflects the combination of weed control by herbicides and the selectivity of herbicides for the crop. Therefore, treatment efficacy together with selectivity for cultivated plants will result in higher crop yields.

In the second experiment, there were significant treatment effects only on the phytotoxicity scores at 13 and 35 DAA.

### Table 3. Phytotoxicity scores determined at 33, 68, and 111 days after application (DAA) of herbicide in the sugarcane crop and sugarcane stalk number and yield at 249 DAA (1st experiment, herbicide applied on Sept. 24th, 2011)

| Herbicide Treatment | Dose (g ha⁻¹) | Phytotoxicity (%) by DAA | Number (stems ha⁻¹) | Yield (tons ha⁻¹) |
|---------------------|---------------|--------------------------|---------------------|------------------|
| Clomazone           | 1200          | 5.00 ab                  | 33                  | 56.67 ab         |
| Clomazone +         | 1200          | 2.50 a                   | 68                  | 62.50 ab         |
| Tebuthiuron          | 325           | 0.00 a                   | 111                 | 63.33 ab         |
| Clomazone +         | 1200          | 22.50 b                  | 249                 | 71.46 a          |
| Hexazinone          | 200           | 0.00 a                   | 265                 | 69.86 a          |
| Clomazone +         | 1200          | 0.00 a                   | 250                 | 0.00             |
| Imazapyr            | 250           | 0.00 a                   | 249                 | 62.92 ab         |
| Clomazone +         | 1200          | 0.00 a                   | 1050                | 62.59 ab         |
| Flumioxazin         | 1200          | 0.00 a                   | 225                 | 69.41 a          |
| Clomazone +         | 1200          | 7.50 a                   | 1507.5              | 70.42 a          |
| Isoxaflutole        | 425           | 6.25 a                   | 425                 | 70.10 a          |
| Sulfometuron +      | 38.25         | 0.00 a                   | 10                   | 39.79 b          |
| Diuron +            | 1507.5        | 0.00 a                   | 250                 | 32.62 b          |
| Hexazinone          | 425           | 0.00 a                   | 225                 | 75.42 a          |
| Hand-weeded control | 0             | 0.00 a                   |                      | 75.04 a          |

** Significant at the 1% probability level using the ANOVA F-test.

Means within columns followed by the same letter do not differ significantly by Tukey’s test at the 5% probability level.

Coefficient of variability.

** Significant at the 5% probability level using the ANOVA F-test.

Means within columns followed by the same letter do not differ significantly by Tukey’s test at the 5% probability level.

Coefficient of variability.

** Least significant difference.

### Table 4. Phytotoxicity score determined at 13, 35, and 59 days after application (DAA) of herbicide in the sugarcane crop and sugarcane stalk number and yield at 265 DAA (2nd experiment, herbicide applied on Oct. 15th, 2011)

| Herbicide Treatment | Dose (g ha⁻¹) | Phytotoxicity (%) by DAA | Number (stems ha⁻¹) | Yield (tons ha⁻¹) |
|---------------------|---------------|--------------------------|---------------------|------------------|
| Clomazone           | 1200          | 12.50 bcd (3)           | 13                  | 88.75 a          |
| Clomazone +         | 1200          | 7.50 ab                 | 35                  | 92.87 a          |
| Tebuthiuron          | 800           | 6.25 abc                | 59                  | 94.61 a          |
| Clomazone +         | 1000          | 13.75 cd                | 112.5               | 88.55 a          |
| Hexazinone          | 250           | 5.00 ab                 | 29                  | 86.28 a          |
| Clomazone +         | 1000          | 16.25 d                 | 1206                | 80.28 a          |
| Imazapyr            | 340           | 10.00 b                 | 325                 | 79.30 a          |
| Flumioxazin         | 700           | 10.00 b                 | 750                 | 93.46 a          |
| Clomazone +         | 1000          | 10.00 b                 | 360                 | 93.46 a          |
| Hexazinone          | 1000          | 0.00 a                  | 500                 | 87.52 a          |
| Hand-weeded control | -             | 0.00 a                  | 13                  | 0.70             |

** Significant at the 1% probability level using the ANOVA F-test.

Means within columns followed by the same letter do not differ significantly by Tukey’s test at the 5% probability level.

Coefficient of variability.

** Least significant difference.
At 13 DAA, the treatment clomazone + amicarbazone caused visible damage (16%) to the sugarcane plants and did not differ from clomazone (12%), clomazone + imazapyr (14%), and sulfometuron + hexazinone + diuron (10%). At 35 DAA, the phytotoxicity scores were generally lower and considered mild (<10%). By the subsequent evaluation, the plants had recovered, and no phytotoxicity symptoms were observed.

There was no significant difference among the treatments for the number or the yield of stalks. These results indicate that the herbicide treatments did not affect the development of the sugarcane plants and that the low infestation by *R. exaltata* did not significantly reduce the number or the yield of stalks, even when the weeds coexisted throughout their lifecycle with the sugarcane crop.

### Conclusion

Under conditions of low (6.1 plants m⁻²) infestation by *R. exaltata*, there was no difference among herbicides. With increased infestation (19.4 plants m⁻²), treatments with clomazone + imazapyr, clomazone + isoxaflutole, and sulfometuron + diuron + hexazinone were superior, with better weed control, as reflected by the seed bank of this weed in soil and sugarcane stalk yield.

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