

HPPD-Inhibiting Herbicides Alone or in Tank-Mix with Atrazine in Elephant Grass

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Abstract

The interference imposed by weeds is one of the most important factors limiting elephant grass forage yield. Two experiments were carried out in 2015/2016 and 2017 to evaluate the selectivity and weed control of 4-hydroxyphenylpyruvate dioxygenase (HPPD)-inhibiting herbicides applied alone or in combination with atrazine in elephant grass. The treatments applied in the experiment conducted in Valença, Rio de Janeiro State, Brazil, were as follows: two rates of mesotrione (0.072 and 0.144 kg ha⁻¹ + 0.5% v/v mineral oil - Assist®), two rates of tembotrione (0.075 and 0.100 kg ha⁻¹ + 0.5% v/v mineral oil - Aureo®), atrazine + mesotrione (1.25 + 0.072 kg ha⁻¹ + 0.5% v/v mineral oil - Assist®), atrazine + tembotrione (1.25 + 0.100 kg ha⁻¹ + 0.5% v/v mineral oil - Aureo®), atrazine + mesotrione (1.25 + 0.072 kg ha⁻¹), atrazine + tembotrione (1.25 + 0.100 kg ha⁻¹) and two checks (weed-free check and weedy check). The same herbicide treatments and a check without application were applied in an experiment conducted in Coronel Pacheco, Minas Gerais State, Brazil. Two application rates of mesotrione with the addition of mineral oil or the tank mixture of atrazine plus mesotrione, with or without the addition of mineral oil, did not provide injuries capable to reduce elephant grass forage yield. Tembotrione was phytotoxic to elephant grass when applied with mineral oil. Atrazine plus tembotrione in a tank-mix, with or without mineral oil, were also phytotoxic to elephant grass. All treatments provided satisfactory weed control.

Keywords: forage, Napier grass, pasture, *Pennisetum purpureum*, weeds

1. Introduction

The majority of Brazil's milk and meat production is based on the use of pasture. Thus, the search for forage species with high productivity and forage quality for cattle feeding has great importance. Elephant grass (*Pennisetum purpureum* Schum.) is suitable for this purpose and widely used in cattle raising, mainly for cutting, grazing and silage production (Santos et al., 2013; Maia et al., 2015). In addition to being an excellent animal feed, elephant grass can also be used as raw material for the production of bio-gas, bio-oil, charcoal (Strezov et al., 2008; Rocha et al., 2017), and alcohol (Shakil et al., 2013), as well as for the generation of electricity (Ohimain et al., 2014).

Elephant grass is known for high productive potential in tropical and subtropical regions; when properly managed, it can produce from 30 to 50 t/ha/year of dry matter (Pereira et al., 2016). In addition, elephant grass is resistant to drought, diseases and pests (Schmelzer, 1997).

However, one of the major limitations on the implantation and conduct of elephant grass fields is related to weed interference (Brighenti et al., 2017a). There is a lack of research on weed management in elephant grass, as most studies consider this species to be a weed rather than a crop (Cutts et al., 2011; Odera & Gilbert, 2012; Grey et al., 2015).

Proper weed management during elephant grass implantation is very important, because the elephant grass crop is very sensitive in its early stages of growth (3-6 weeks after planting) (Brighenti et al., 2017b). Normally, elephant grass is installed during the rainy season in Brazil (November to January), which coincides with high temperatures, favoring the emergence and establishment of various weeds, especially grasses. These weeds,

mainly *Brachiaria* species, demand special attention by growers at the moment of elephant grass crop implantation and conduction (Abreu et al., 2006).

Herbicides are the most commonly used method to control weeds. Carotenoid biosynthesis inhibitor herbicides, particularly those that inhibit the HPPD-enzyme, are noteworthy and commonly used on corn (Choe et al., 2014; Stephenson et al., 2015). HPPD-inhibiting herbicides have become popular among corn growers because of their broad-spectrum of weed control, flexible application timing, tank-mix compatibilities, and crop safety (Bollman et al., 2008; Walsh et al., 2012).

Another herbicide widely used on corn is atrazine (Rodrigues & Almeida, 2011). It can be applied to a crop before, during or after planting of the seeds, or even after crop emergence. It is most often used at low rates in tank-mixes to improve broad-spectrum weed control (Bollman et al., 2008).

The development of methods for weed control by means of selective herbicides is an important practice in order to expand elephant grass cultivation in tropical and subtropical regions.

The objectives of this work were to evaluate the selectivity and weed control of HPPD-inhibiting herbicides alone or in combination with atrazine in elephant grass.

2. Method

2.1 Study Sites

Two experiments were conducted in experimental areas in the municipalities of Valença, Rio de Janeiro State, Brazil (22°22'06.53"S, 43°42'00.48"W; Experiment 1) and Coronel Pacheco, Minas Gerais State, Brazil (21°33'11.10"S, 43°15'56.32"W; Experiment 2).

2.2 Experimental Implantations

Experiment 1 was implanted under field conditions in a soil classified as Red-Yellow Argisol. The samples were collected at 0-20 cm depth and the results of the chemical and textural analyses were as follows: pH (H₂O) = 5.1, P = 9.6 mg dm⁻³, K = 90 mg dm⁻³, Ca²⁺ = 2.4 cmol_c dm⁻³, Mg²⁺ = 2.3 cmol_c dm⁻³, Al³⁺ = 0.0 cmol_c dm⁻³, H + Al = 3.3 cmol_c dm⁻³, SB = 4.93 cmol_c dm⁻³, CEC (t) = 4.93 cmol_c dm⁻³, CEC (T_{pH=7.0}) = 8.23 cmol_c dm⁻³, V = 60%, C organic = 1.94 dag kg⁻¹, clay = 18%, silt = 16%, sand = 66%.

Experiment 1 was installed on October 30, 2015. Before elephant grass planting, the experimental area was plowed and barred. Furrows 0.2 m deep, with spacing of 1.0 m between rows, were fertilized with 200 kg ha⁻¹ of MAP (mono-ammonium phosphate). Stem cuttings of elephant grass cultivar 'BRS Capiacu', 40 cm long with four buds per cutting, were planted in the furrows and covered with approximately 15 cm of soil. Each plot was four furrows (rows) wide by 5 m long (20 m²). Plots were side-dressed with 40 kg of N ha⁻¹ (urea) 30 days after planting.

The herbicide doses were applied on November 19, 2015, when the elephant grass plants were approximately 0.25 m tall. All herbicides were applied using a backpack sprayer (Herbicat Ltda, Catanduva, São Paulo State, Brazil), CO₂ pressurized (2 kgf cm⁻²) to deliver a volume equivalent to 140 L ha⁻¹. The sprayer boom (1.5 m length) comprised four flat fan nozzles (Magnojet BD 110 02), spaced 0.5 m apart. The environmental conditions during herbicide spraying were temperature, 27 °C; relative humidity, 68%; and wind speed, 2 m s⁻¹.

The predominant weed species and their densities at the time of herbicide applications were: *Brachiaria plantaginea* (3.0 plants m⁻²), *Ipomoea cordifolia* (2.0 plants m⁻²), *Cyperus esculentus* (4.0 plants m⁻²) and *Commelina benghalensis* (3.0 plants m⁻²).

Experiment 2 was installed on May 25, 2017, in Coronel Pacheco. Containers of 5 kg capacity were filled with a substrate containing equal quantities of sand, manure and soil. Three elephant grass (cultivar 'BRS Capiacu') stem cuttings (10 cm long) were planted in containers arranged on benches and directly exposed to the sun (under field conditions). Weekly irrigations were performed in order to maintain the substrate in the field capacity. After emergence, two plants were kept per container.

The treatments were applied on June 22, 2017, using the same sprayer and calibration mentioned above. The environmental conditions during the herbicide spraying were temperature, 22 °C; relative humidity, 66%; and wind speed, 3 m s⁻¹. At that time, plants were 0.25 m tall on average.

2.3 Treatments and Experimental Designs

A completely randomized block design with four replicates was used in Experiment 1. The treatments applied were as follows: two rates of mesotrione (0.072 and 0.144 kg ha⁻¹ + 0.5% v/v mineral oil - Assist®), two rates of tembotrione (0.075 and 0.100 kg ha⁻¹ + 0.5% v/v mineral oil - Aureo®), atrazine plus mesotrione (1.25 + 0.072

kg ha⁻¹ + 0.5% v/v mineral oil - Assist®), atrazine plus tembotrione (1.25 + 0.100 kg ha⁻¹ + 0.5% v/v mineral oil - Aureo®), atrazine plus mesotrione (1.25 + 0.072 kg ha⁻¹), atrazine plus tembotrione (1.25 + 0.100 kg ha⁻¹) and two checks [weed-free check (manual weed control) and weedy check (without manual weed control)]. Experiment 2 was arranged in a completely randomized design with four replications. The same herbicidal treatments of Experiment 1 were applied, plus a check without herbicide application.

2.4 Measurements of Elephant Grass and Weeds

Phytotoxic effects of herbicide treatments on the elephant grass and the weed control were evaluated on a scale of 0% to 100% at 10, 20 and 30 days after treatments (DAT). The value zero corresponded to no symptoms of phytotoxicity on elephant grass plants or no weed control, and 100% to complete elephant grass death or complete weed control (SBCPD, 1995).

All weed species were cut on the soil surface within a quadrat (0.5 × 0.5 m) to determine aboveground fresh matter weight at 30 DAT.

For both experiments, green color indices were evaluated on elephant grass leaves at 23 DAT using a SPAD-502 chlorophyll meter (Konica Minolta, Japan). Simultaneously with the SPAD index evaluation, the elephant grass plant height was determined in Experiment 2 using a graduated ruler.

The harvest and determination of forage yield were performed on February 15, 2016, approximately 110 days after elephant grass establishment (Experiment 1). Plants were cut within a quadrat (1.0 × 1.0 m) at the soil surface to quantify the aboveground biomass. Harvested plants were weighed, placed in a forced-air ventilation oven at 65 °C for 72 h; and subsequently reweighed. The values of fresh and dry matter weight of elephant grass were converted to kg ha⁻¹.

In Experiment 2, the harvest was carried out on July 13, 2017. The plants were cut at the soil surface in order to obtain the aboveground biomass per container. The plants were placed in paper bags and dried using the same procedure described in Experiment 1. The dried matter weights were then recorded.

2.5 Statistical Analysis

The percentage of phytotoxicity for both experiments and the weed control percentages of Experiment 1 were normalized by square root transformation of (x + 1) to perform analysis of variance tests. Data were subjected to analyses of variance, and the mean values were compared using the Scott-Knott test ($P \leq 0.05$). Statistical analyses were performed using SAEG software (Ribeiro Júnior, 2001).

2.6 Climate Conditions

The mean monthly air temperatures (mean, maximum and minimum) and rainfall during the experimental periods are shown in Table 1.

Table 1. Average maximum and minimum monthly air temperatures (T), and rainfall during experiments conducted at Valença, Rio de Janeiro State, and Coronel Pacheco, Minas Gerais State, Brazil

Municipality	Valença					Coronel Pacheco		
	Oct/15	Nov/15	Dec/15	Jan/16	Feb/16	May/17	Jun/17	Jul/17
Maximum T (°C)	29.2	29.9	31.6	29.9	32.6	22.2	21.7	20.0
Minimum T (°C)	20.8	20.7	20.7	20.5	20.7	21.0	20.2	18.5
Rainfall (mm)	75.3	118.8	141.4	388.2	222.4	68.4	23.6	7.0

3. Results and Discussion

The application of two rates of mesotrione with the addition of mineral oil or the tank mixture of atrazine plus mesotrione, with or without the addition of mineral oil, were highly selective to elephant grass plants (Tables 2 and 3). In both experiments, those treatments presented 0% phytotoxicity, at 30 DAT.

The response of millet (Cultivar 'ADR-300'), submitted to the dose of 60 g ha⁻¹ of mesotrione, was evaluated by Dias et al. (2015). The results also indicated selectivity of this herbicide to millet, since there was no damage to plant growth. Other studies conducted by Abit et al. (2009) revealed the possibility of using mesotrione in sorghum. Several genotypes were submitted to post-emergence applications at doses of 0, 52, 105, 210 and 315 g ha⁻¹. The results showed 17 mesotrione-tolerant sorghum hybrids, and, when grown under field conditions, the symptoms of injury did not correlate with crop yield. Takano et al. (2016) evaluated mesotrione (50 and 100 g

ha⁻¹) and the mixture of atrazine plus mesotrione (1000 + 50 g ha⁻¹) in six sorghum hybrids. These treatments were selective for the sorghum crop, demonstrating that they were an option for post-emergence application.

The two doses of tembotrione with the addition of mineral oil caused total death of the plants, already in the second evaluation, at 20 DAT (Experiment 1, Table 2). These values were also high and varied from 87% to 94% at 30 DAT in Experiment 2 (Table 3).

Table 2. Percentage of phytotoxicity on elephant grass plants at 10, 20 and 30 days after application of the treatments. Valença. Rio de Janeiro State, Brazil. (Experiment 1)

Treatments	Doses (kg ha ⁻¹)	Days after treatment		
		10	20	30
Mesotrione	0.072 + 0.5% oil	0.0 D ¹	0.0 D	0.0 C
Mesotrione	0.144 + 0.5% oil	0.0 D	0.0 D	0.0 C
Tembotrione	0.075 + 0.5% oil	98.0 A	100.0 A	100.0 A
Tembotrione	0.100 + 0.5% oil	98.2 A	100.0 A	100.0 A
Atrazine + Mesotrione	1.25 + 0.072 + 0.5% oil	0.0 D	0.0 D	0.0 C
Atrazine + Tembotrione	1.25 + 0.100 + 0.5% oil	87.7 B	98.0 B	100.0 A
Atrazine + Mesotrione	1.25 + 0.072	0.0 D	0.0 D	0.0 C
Atrazine + Tembotrione	1.25 + 0.100	14.7 C	54.2 C	65.0 B
Weed-free Check	-	0.0 D	0.0 D	0.0 C
Weedy Check	-	0.0 D	0.0 D	0.0 C
Coefficient of Variation (%)	-	1.2	0.8	0.3

Note. ¹ Mean values followed by different letters are significantly different ($P \leq 0.05$) by Scott-Knott.

Table 3. Percentage of phytotoxicity on elephant grass plants at 10, 20 and 30 days after application of the treatments. Coronel Pacheco. Minas Gerais State. Brazil. (Experiment 2)

Treatments	Doses (kg ha ⁻¹)	Days after treatment		
		10	20	30
Mesotrione	0.072 + 0.5% oil	0.0 H ¹	0.0 E	0.0 E
Mesotrione	0.144 + 0.5% oil	1.6 F	0.6 E	0.0 E
Tembotrione	0.075 + 0.5% oil	40.6 C	80.3 C	87.3 C
Tembotrione	0.100 + 0.5% oil	70.3 B	85.6 B	94.0 B
Atrazine + Mesotrione	1.25 + 0.072 + 0.5% oil	5.6 E	2.6 E	0.0 E
Atrazine + Tembotrione	1.25 + 0.100 + 0.5% oil	83.0 A	91.6 A	100.0 A
Atrazine + Mesotrione	1.25 + 0.072	0.6 G	0.3 E	0.0 E
Atrazine + Tembotrione	1.25 + 0.100	12.3 D	58.3 D	61.6 D
Check	-	0.0 H	0.0 E	0.0 E
Coefficient of Variation (%)	-	2.9	3.1	0.9

Note. ¹ Mean values followed by different letters are significantly different ($P \leq 0.05$) by Scott-Knott test.

Characteristic symptoms were the appearance of bleached leaves and stunted plants. Herbicides of this chemical group inhibit the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), acting in the conversion of tyrosine to plastoquinone and α -tocopherol. The inhibition of plastoquinone leads to an interruption of the carotenoid synthesis pathway (Oliveira Júnior, 2011). The blocking synthesis of these pigments is responsible for the characteristic symptom of depigmentation or “albinism” due to the lack of chlorophyll protection from photodegradation. Growth ceases in absence of the production of green photosynthetic pigments and symptoms of necrosis and plant death begin to appear. Tembotrione (100.8 g ha⁻¹) was applied alone and in a tank mix with atrazine (1,500 g ha⁻¹) on three sorghum cultivars (Galon et al., 2016). These treatments were highly detrimental to cultivars, with percentages of phytotoxicity varying from 98% to 100%.

Atrazine and tembotrione in a tank-mix plus mineral oil provided high levels of phytotoxicity and plant death at 30 DAT (100%) (Tables 2 and 3). Reductions in the percentage of phytotoxicity were observed for atrazine plus

tembotrione without mineral oil, ranging from 12% to 65% in both experiments. However, these values were still high and resulted in significant forage yield loss (Tables 4 and 5).

Table 4. SPAD indices (SPAD) on elephant grass plants, fresh matter weight (FMW) (kg ha^{-1}) and dry matter weight (DMW) (kg ha^{-1}) of elephant grass. Valença, Rio de Janeiro State, Brazil. (Experiment 1)

Treatments	Doses (kg ha^{-1})	SPAD	FMW	DMW
Mesotrione	0.072 + 0.5% oil	56.3 A ¹	159.300.0 A	44.720.19 A
Mesotrione	0.144 + 0.5% oil	56.2 A	177.125.0 A	43.239.14 A
Tembotrione	0.075 + 0.5% oil	18.4 D	53.800.0 C	14.598.98 C
Tembotrione	0.100 + 0.5% oil	30.4 C	64.850.00 C	17.496.23 C
Atrazine + Mesotrione	1.25 + 0.144 + 0.5% oil	53.3 A	165.750.0 A	46.533.98 A
Atrazine + Tembotrione	1.25 + 0.100 + 0.5% oil	30.9 C	79.400.0 C	21.460.32 C
Atrazine + Mesotrione	1.25 + 0.144	53.9 A	180.050.0 A	49.079.70 A
Atrazine + Tembotrione	1.25 + 0.100	46.7 B	119.600.0 B	34.635.12 B
Weed-free Check	-	53.4 A	163.600.0 A	44.557.86 A
Weedy Check	-	50.1 B	69.600.0 C	19.920.53 C
Coefficient of Variation (%)	-	7.1	12.8	17.9

Note. ¹ Mean values followed by different letters are significantly different ($P \leq 0.05$) by Scott-Knott test.

Table 5. SPAD indices (SPAD), plant height (H) (cm), fresh (FMW) and dry matter weight (DMW) (g container^{-1}) of elephant grass. Coronel Pacheco, Minas Gerais State, Brazil. (Experiment 2)

Treatments	Doses (kg ha^{-1})	SPAD	H	FMW	DMW
Mesotrione	0.072 + 0.5% oil	28.5 A ¹	60.1 A	98.5 A	22.7 A
Mesotrione	0.144 + 0.5% oil	28.9 A	58.7 A	109.4 A	23.9 A
Tembotrione	0.075 + 0.5% oil	13.0 B	43.3 B	53.3 B	17.3 B
Tembotrione	0.100 + 0.5% oil	11.4 B	40.0 B	36.6 B	15.6 B
Atrazine + Mesotrione	1.25 + 0.072 + 0.5% oil	27.8 A	56.6 A	97.3 A	22.2 A
Atrazine + Tembotrione	1.25 + 0.100 + 0.5% oil	0.0 C	0.0 C	17.3 C	14.9 B
Atrazine + Mesotrione	1.25 + 0.072	28.1 A	56.6 A	96.6 A	22.5 A
Atrazine + Tembotrione	1.25 + 0.100	13.0 B	36.6 B	42.5 B	16.9 B
Check	-	28.6 A	56.7 A	106.0 A	24.0 A
Coefficient of Variation (%)	-	5.5	7.2	12.7	5.9

Note. ¹ Mean values followed by different letters are significantly different ($P \leq 0.05$) by Scott-Knott test.

All treatments of mesotrione plus mineral oil or a tank mixture of atrazine plus mesotrione, with or without the addition of mineral oil, resulted in SPAD index values statistically equal to the weed-free check (Experiment 1) and the check without application (Experiment 2) (Tables 4 and 5, respectively). This result was also verified for elephant grass plant height in Experiment 2 (Table 5). Mesotrione plus mineral oil or a tank mixture of atrazine plus mesotrione, with or without the addition of mineral oil did not reflect in forage yield losses, where values of fresh and dry matter weights were similar to those reached in the weed-free check and in the check without application (Tables 4 and 5, respectively).

On the other hand, treatments with tembotrione plus mineral oil and atrazine plus tembotrione with and without mineral oil resulted in low SPAD index values (Tables 4 and 5). This fact reinforced the results obtained with visual evaluations of phytotoxicity. Elephant grass growth and development, which were adversely affected which reflected in forage yield losses.

Even though tembotrione has been registered for corn in post-emergence applications (Rodrigues & Almeida 2011; Idziak & Woznica, 2014), some sweet corn genotypes are sensitive to it (Bollman et al., 2008). The corn hybrid 'Merit' cultivar presents low tolerance to tembotrione applied alone or in a tank mixture with atrazine.

Weed interference in elephant grass can be measured when comparing the two checks (Table 4). The reduction of elephant grass fresh and dry matter weights was evident as a result of weed interference. The weedy check

yielded 42.5% and 44.7% significantly less fresh and dry matter weights when compared to the weed-free check, respectively.

Weed control in all mesotrione treatments ranged from 77% to 87% at 30 DAT (Table 6).

Table 6. Percentage of weed control on elephant grass plants at 10, 20 and 30 days after application of the treatments (DAT) and fresh matter weight of weed (g 0.25 m⁻²) (FMW₁) at 30 DAT. Valença, Rio de Janeiro State, Brazil. (Experiment 1)*

Treatments	Doses (kg ha ⁻¹)	Days after treatment			FMW ₁
		10	20	30	
Mesotrione	0.072 + 0.5% oil	62.5 D ¹	50.0 E	77.2 E	1.47 B
Mesotrione	0.144 + 0.5% oil	78.2 C	62.5 C	87.2 C	0.90 B
Tembotrione	0.075 + 0.5% oil	98.7 A	99.5 A	100.0 A	0.62 B
Tembotrione	0.100 + 0.5% oil	99.5 A	100.0 A	100.0 A	1.04 B
Atrazine + Mesotrione	1.25 + 0.072 + 0.5% oil	57.2 E	50.2 E	85.0 D	1.01 B
Atrazine + Tembotrione	1.25 + 0.100 + 0.5% oil	99.5 A	100.0 A	100.0 A	0.61 B
Atrazine + Mesotrione	1.25 + 0.072	60.7 D	58.2 D	84.7 D	0.98 B
Atrazine + Tembotrione	1.25 + 0.100	87.2 B	89.2 B	95.5 B	0.96 B
Weed-free Check	-	100.0 A	100.0 A	100.0 A	0.26 B
Weedy Check	-	0.0 F	0.0 F	0.0 F	2.44 A
Coefficient of Variation (%)	-	1.8	0.9	0.5	54.2

Note.* Weed species were as follows: *Brachiaria plantaginea*, *Ipomoea cordifolia*, *Cyperus esculentus* and *Commelina benghalensis*. ¹Mean values followed by different letters are significantly different ($P \leq 0.05$) by Scott-Knott test.

Although the lowest dose of this herbicide resulted in 77% weed control, there was no loss on forage yield in function of weed interference. The addition of atrazine to mesotrione provided improvements in weed control. The percentage of weed control, at 30 DAT, ranged from 77% (mesotrione: 0.072 + 0.5% oil) to 85% (atrazine + mesotrione: 1.25 + 0.072 + 0.5% oil). Williams et al. (2011) emphasized the importance of using atrazine in a tank-mix with other herbicides due to the increase in spectrum of weed control. The authors observed that in tank mixing of atrazine with HPPD-inhibiting herbicides provided increases in the number and weight of ears of sweet corn of 9% and 13%, respectively, when compared to these herbicides applied alone.

The best weed control treatments were tembotrione alone or in combination with atrazine; the percentages ranged from 95% to 100%, at 30 DAT. All herbicide treatments reduced the fresh matter weights of weeds, differing statistically from the weedy check.

4. Conclusions

This research provides alternatives to weed control in elephant grass fields. Different herbicide options that are feasible for use in elephant grass can facilitate increases in cultivated areas and forage supplies, mainly in tropical and subtropical regions.

Two application rates of mesotrione with the addition of mineral oil or the tank mixture of atrazine plus mesotrione, with or without the addition of mineral oil, did not provide injuries capable to reduce elephant grass forage yield. Tembotrione was phytotoxic to elephant grass when applied with mineral oil. Atrazine plus tembotrione in a tank-mix, with or without mineral oil, were also phytotoxic to elephant grass. All treatments provided satisfactory weed control.

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