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Article

# Ammonium Glufosinate Associated with Post-Emergence Herbicides in Corn with the *cp4-epsps* and *Pat* Genes

*Amônio-Glufosinate Associado com Herbicidas em Pós-Emergência no Milho com os Genes **cp4-epsps** e **Pat** Genes*

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ABSTRACT - With the possibility of using ammonium glufosinate in corn, studies on its association with other herbicides are essential. The aim of this study was to assess weed control and herbicide selectivity in association with ammonium glufosinate in corn containing the *pat* and *cp4-epsps* genes. The experiment was carried out under field conditions in two sites in a randomized block design with four replications. Treatments consisted of ammonium glufosinate, glyphosate, ammonium glufosinate + glyphosate, ammonium glufosinate + nicosulfuron, ammonium glufosinate + atrazine, ammonium glufosinate + tembotrione, ammonium glufosinate + mesotrione, ammonium glufosinate + carfentrazone ethyl, ammonium glufosinate + bentazon, ammonium glufosinate + 2,4-D, control without weeding, and hand-weeded control. Visual assessments were performed regarding damage, electron transport rate in photosystem II (ETR), ammonia quantification, height, and grain yield of corn plants, as well as weed control at both sites. The application of ammonium glufosinate and its associations did not provide a reduction in productivity when compared to the hand-weeded control. Some treatments presented higher levels of visual damage and ammonia accumulation, but without a yield reduction. Therefore, herbicide mixture with ammonium glufosinate may be an alternative to weed control without damaging corn crop, which presents in its genetic constitution the *cp4-epsps* and *pat* genes.

**Keywords:** ammonia accumulation, phosphinothricin, electron transport rate, herbicide resistance, *Zea mays*.

*RESUMO - Com a possibilidade do uso de amônio-glufosinate no milho, são fundamentais estudos com associações desse herbicida junto a outros. O objetivo do presente trabalho foi avaliar o controle de plantas daninhas e a seletividade de herbicidas em associação ao amônio-glufosinate em milho contendo os genes **pat** e **cp4-epsps**. O experimento foi realizado em campo em duas localidades, dispostos em blocos ao acaso com quatro repetições. Os tratamentos foram: amônio-glufosinate; glyphosate; amônio-glufosinate + glyphosate; amônio-glufosinate + nicosulfuron; amônio-glufosinate + atrazine; amônio-glufosinate + tembotrione; amônio-glufosinate + mesotrione; amônio-glufosinate + carfentrazone ethyl; amônio-glufosinate + bentazon; amônio-glufosinate + 2,4-D; e testemunha sem capina e testemunha capinada. Foram feitas realizadas avaliações visuais de injúria, fluxo de transporte de elétrons do fotossistema II (ETR), quantificação de amônia, altura e rendimento de grãos das plantas de milho, além de controle das plantas daninhas em ambos os locais. A adoção da aplicação de glufosinate e suas associações não proporcionou redução de produtividade quando comparada à testemunha capinada; alguns tratamentos apresentaram maiores níveis de injúria*

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*visual e acúmulo de amônia, porém sem redução de produtividade. Portanto, a mistura de herbicidas com o amônio-glufosinate pode ser uma alternativa para o controle de plantas daninhas, sem causar danos à cultura do milho, que apresenta na sua constituição genética os genes* ***cp4-epsps*** *e* ***pat****.*

**Palavras-chave:** acúmulo de amônia, fosfinotricina, fluxo de transporte de elétrons, resistência a herbicidas, *Zea mays*.

## INTRODUCTION

Corn is of great importance in the Brazilian agriculture scenario. An area of 17 million hectares was sown in the 2016/17 season, with an estimated production of 91 million tons and an average yield of 5,000 kg $ha^{-1}$, considering the first and second harvests (Conab, 2017). In this sense, transgenic had and has an important role in the success of corn cultivation in Brazil. In the last 10 years, more than 40 transgenic events, among them technologies that guarantee insect resistance and resistance to herbicides, have been released in the market.

A number of factors can impair corn production, and one of the most important is weed interference. Weeds can cause losses from 13 to 88% in corn yield (Dan et al., 2010). In this scenario, the genetically modified corn presents events that guarantee resistance to the herbicides glyphosate, ammonium glufosinate, 2,4-D, and haloxyfop-methyl, facilitating and improving weed control.

The herbicide ammonium glufosinate is derived from phosphinothricin, a toxin isolated from the fungi *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* (Dayan et al., 2009; Dayan and Duke, 2014). It is a non-selective herbicide and acts by inhibiting the enzyme glutamine synthetase (GS) activity, which has the function of ammonia detoxification and production of the amino acid glutamine (Barnett et al., 2012). Plants susceptible to ammonium glufosinate exhibit glutamine deficiency, poisoning by ammonia accumulation, glutamate and glyoxalate accumulation, rupture of chloroplast structure, decreased electron transport, and inhibition of photosynthesis (Kleczkowski, 1993; Coetzer and Al-Khatib, 2001; Dayan et al., 2015; Carbonari et al., 2016).

Corn tolerance to ammonium glufosinate is linked to the insertion of the *pat* gene from *Streptomyces viridochromogenes*, which, when transcribed in the plant, produces the enzyme phosphinothricin acetyltransferase (PAT), which has the ability to chemically inactivate the herbicide ammonium glufosinate in N-acetyl-L-glufosinate (Droge-Laser et al., 1994), which does not generate GS insensitivity (Hérouet et al., 2005; Tan et al., 2006). The *pat* gene was used to serve as a selective marker in corn, with its expression associated with insect resistance genes. However, under this condition, the herbicide ammonium glufosinate can be used in corn without significant damage to crop productivity (Armel et al., 2008; Silva et al., 2017).

A problem of using genetically modified corn tolerant to herbicide is its indiscriminate use because herbicides such as glyphosate and ammonium glufosinate have a broad spectrum of action and high efficiency in weed control. In turn, the overuse of these herbicides in successive agricultural seasons may result in the selection of resistant weeds. According to López-Ovejero et al. (2006), the selection of weeds resistant to herbicides in agricultural crops both nationally and internationally is one of the main problems related to weed control. In this context, the association of herbicides has a great potential of use mainly due to an increase in the spectrum of control, which includes weeds that already show resistance or tolerance to herbicides, thus assisting in reducing the expansion and prevention of resistance (Owen and Zelaya, 2005).

The association of herbicides has been studied in corn cultivation, especially glyphosate in association with nicosulfuron, atrazine, 2,4-D, bentazon, and tembotrione or even among associations of the latter herbicides (Jakelaitis et al., 2007; Rezende et al., 2014; Vieira Junior et al., 2015; Osório et al., 2015). Armel et al. (2008) assessed the effect of ammonium glufosinate with mesotrione whereas Silva et al. (2017) assessed ammonium glufosinate in association with glyphosate and atrazine. Both studies were conducted in corn containing the *pat* gene and no significant yield losses were observed.

Considering there are few studies on mixtures with ammonium glufosinate for weed control in corn and the commercial availability of technologies that allow the application of this herbicide, new researches should be carried out with the use of ammonium glufosinate in association with other herbicides in corn with the *pat* gene. Thus, this study aimed to assess weed control and herbicide selectivity in association with ammonium glufosinate in corn containing the *pat* and *cp4-epsps* genes.

## MATERIAL AND METHODS

The experiments were conducted under field at two sites: Botucatu and São Manoel, SP, Brazil. Soil chemical characteristics of each site are shown in Table 1. A liming was conducted prior to corn sowing aiming at raising base saturation to 60%, and then a heavy harrowing was performed. A fertilization with the NPK formulation 08-28-16 was carried out at both sites at sowing time. Liming and fertilization were carried out considering the soil analysis.

The experimental design was a randomized block design with four replications. Herbicide treatments, their respective doses, and commercial products are shown in Table 2. We used the Dow AgroSciences corn simple hybrid 2A401, which presents the Cry1A.105, Cry2Ab2 and Cry1F genes, which have resistance to insects (Milho Power core®) and resistance to glyphosate and ammonium glufosinate due to the presence of the *cp4-epsps* and *pat* genes. This hybrid was sown in both sites at a density of 60 thousand seeds ha$^{-1}$, spaced 0.85 m between rows. Seeds were treated with the commercial mixture of pyraclostrobin + fipronil (Standak Top®) at a dose of 200 mL per 100 kg of seeds. Pest and disease management was not necessary to carry out during the crop cycle.

***Table 1*** - Soil physicochemical characteristics of the experimental area in Botucatu and São Manoel, SP, Brazil

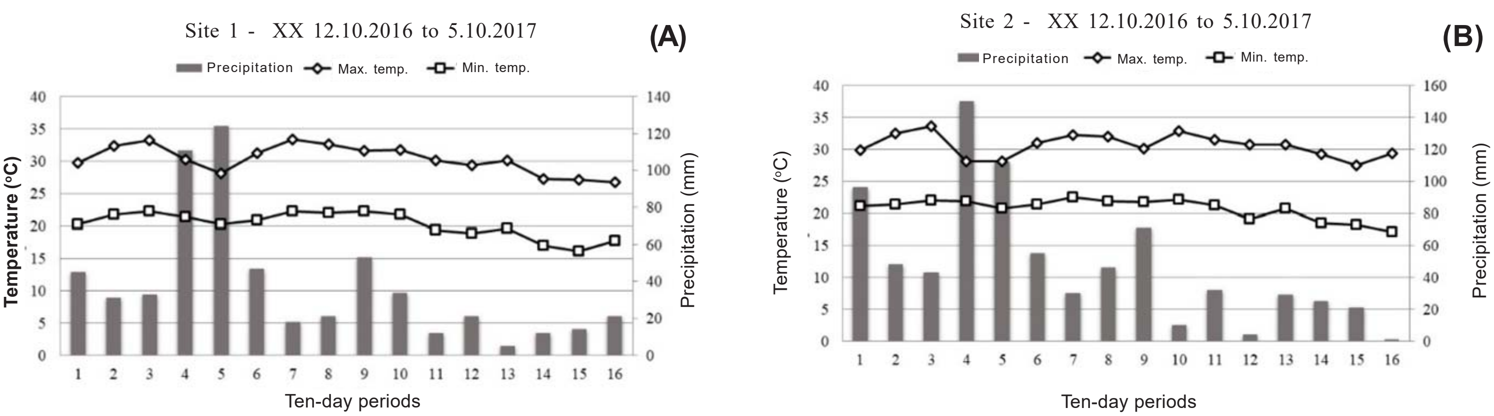
| Site | Chemical property | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | pH ($CaCl_2$) | OM (g dm$^{-3}$) | $P_{resin}$ (mg dm$^{-3}$) | $Al^{3+}$ | K | Ca | Mg | SB | CEC | BS |
| | | | | (mmol$_c$ dm$^{-3}$) | | | | | | |
| Botucatu | 5.1 | 21 | 216 | 1 | 4.3 | 30 | 13 | 48 | 98 | 59 |
| São Manoel | 4.9 | 5 | 27 | 1 | 3.4 | 15 | 6 | 25 | 37 | 50 |

| Site | Physical property | | | |
|---|---|---|---|---|
| | Sand | Silt | Clay | Texture |
| | (g kg$^{-1}$) | | | |
| Botucatu | 195 | 278 | 527 | Clayey |
| São Manoel | 844 | 39 | 117 | Sandy |

***Table 2*** - Treatments used in the experiments with corn in Botucatu and São Manoel, SP, Brazil

| Herbicide | Dose (g a.i. ha$^{-1}$) | Commercial name |
|---|---|---|
| Ammonium glufosinate | 500 | Finale |
| Glyphosate | 960 (g a.e. ha$^{-1}$) | Roundup Ready |
| Ammonium glufosinate + glyphosate | 500 + 960 (g a.e. ha$^{-1}$) | Finale + Roundup Ready |
| Ammonium glufosinate + nicosulfuron | 500 + 60 | Finale + Sanson |
| Ammonium glufosinate + atrazine | 500 + 2400 | Finale + Primóleo |
| Ammonium glufosinate + tembotrione | 500 + 100 | Finale + Soberan |
| Ammonium glufosinate + mesotrione | 500 + 192 | Finale + Callisto |
| Ammonium glufosinate + carfentrazone ethyl | 500 + 30 | Finale + Aurora |
| Ammonium glufosinate + bentazon | 500 + 720 | Finale + Basagran |
| Ammonium glufosinate + 2,4-D | 500 + 1005 (g a.e. ha$^{-1}$) | Finale+ DMA806 |
| Control without weeding | - | - |
| Hand-weeded control | - | - |

Treatment applications were carried out at the phenological stage V4 by using a backpack $CO_2$ pressurized sprayer, with a constant pressure of 2 BAR (or 29 PSI), a flow rate of 0.65 L $min^{-1}$, and equipped with a boom containing six nozzles spaced 50 cm with XR 110.02 (Teejet) flat jet tips. Spraying was performed at a height of 50 cm from the target, at a speed of 1 m $s^{-1}$, and with a spray solution volume of 200 L $ha^{-1}$. The meteorological conditions at the application time were temperatures of 26 and 27 °C, relative air humidity of 60 and 56%, and wind speed of 1.22 and 1.41 km $h^{-1}$ for the application in Botucatu and São Manoel, respectively. The climatic data of maximum and minimum temperature and precipitation obtained during the experimental period are shown in Figure 1.




Source: Brazilian National Institute of Meteorology (INMET).

***Figure 1*** - Precipitation (mm), average minimum temperature, and average maximum temperature (°C) in ten-day periods for the experimental period in the 2016/17 season in Site 1 (A) and Site 2 (B) -12/10/2016 to 05/10/2017, SP Brazil.

A weed survey was carried out and an infestation with wild radish (*Raphanus raphanistrum*), purple nutsedge (*Cyperus rotundus*), alexandergrass (*Urochloa plantaginea*), and Brazil pusley (*Richardia brasiliensis*) was observed at densities of 12 and 20, 23 and 33, 6 and 17, and 14 and 6 plants $m^{-2}$, respectively for each weed and experiments installed in Botucatu and São Manoel, SP, Brazil.

During the experiment, the following variables were assessed: weed control, visual damage, electron transport rate in photosystem II (ETR), ammonia quantification, height, and grain yield of corn plants.

For the assessment of weed control, a visual control score was assigned according to a 0 to 100% control scale, in which 0% means no control and 100% means plant death (SBCPD, 1995). This assessment was performed at 28 days after application (DAA). The assessment of visual damage in corn plants was carried out at 7 and 14 DAA by using the same control scale. Height was assessed in corn plants at 14 DAA and at the R6 stage (physiological maturity) with a graduated ruler by measuring the plants from the soil surface to the last fully expanded leaf for the initial height and until the base of the tassel at R6.

The electron transport rate in photosystem II (ETR) was assessed at 3 h after application and at 1, 2, 3, 5, 7, and 10 DAA by using a portable fluorometer (Multi-Mode Chlorophyll Fluorometer OS5p, Opti Sciences), with readings performed in six plant of each plot at three points on the youngest, fully expanded leaf. The results were expressed as a percentage in relation to the hand-weeded control (considered as 100%).

Ammonia was quantified by the colorimetric method (Wendler et al., 1990; Dayan et al., 2015). Four fully expanded leaves were collected per plant to compose one sample per plot. These samples were conditioned in containers with 300 mL of water acidified with hydrochloric acid (pH 3.5) and submitted to an ultrasonic bath for 60 minutes. The ammonia content present in the solution was determined using a spectrometer (Cintra 40, GBC Scientific Equipment Ltd.) at a wavelength of 630 nm. This analysis was performed at 2 and 4 DAA and data were expressed as mg kg$^{-1}$ fresh matter. The ammonia of treatments to ammonia of hand-weeded control ratio was then calculated.

Grain yield was calculated by harvesting the ears of two central rows with four meters in length, which were threshed in an experiment thresher, and the samples were cleaned with sieves and conditioned in paper bags. Based on grain yield in the plots, yield in kg ha$^{-1}$ was estimated for each treatment and replication. The degree of moisture was corrected to 13% wet basis.

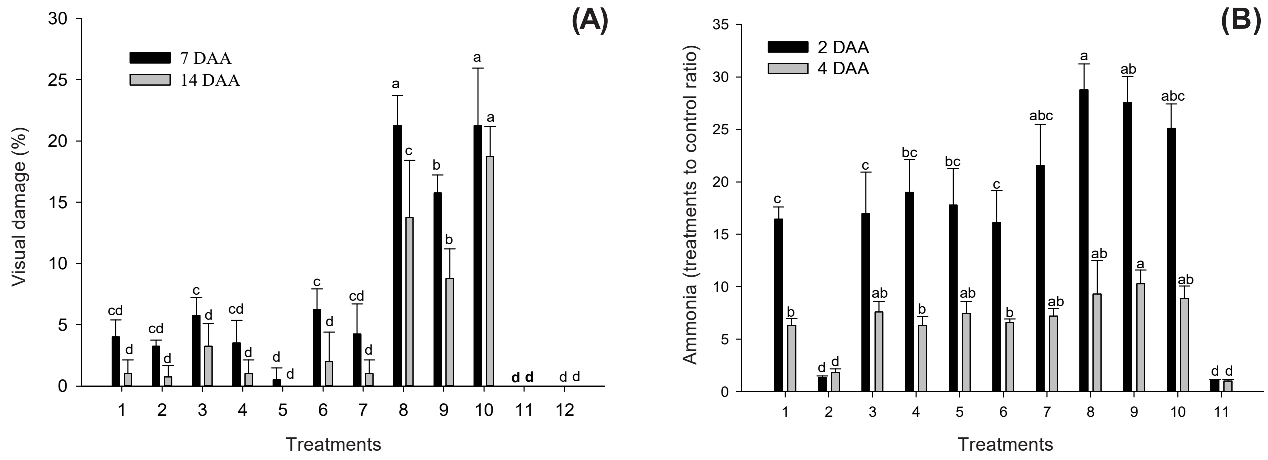
The data were submitted to analysis of variance (ANOVA) and the means were compared by the Tukey's test ($p \leq 0.05$). The confidence interval was calculated for each mean (mean ± confidence interval) of all variables. A joint analysis between the experimental sites was also performed.

## RESULTS AND DISCUSSION

No significant differences were observed among the sites for the variables control of *R. raphanistrum*, *C. rotundus*, *U. plantaginea*, and *R. brasiliensis*, visual damage at 7 and 14 DAA, plant height at 14 DAA, plant height at R6, and ammonia at 2 and 4 DAA. Thus, the data were analyzed and discussed together for both experiments. For the ETR data at 3 h after application and 1, 2, 3, 5, and 7 DAA and productivity, differences were observed between sites (*P-value*: 0.0412, 0.0314, 0.0447, 0.0125, 0.0214, 0.0047, and 0.0128, respectively), which was discussed separately.

Visual damage of corn plants was more pronounced for some associations of herbicides at 7 and 14 DAA in relation to isolated applications. Associations of ammonium glufosinate + carfentrazone ethyl (8), ammonium glufosinate + bentazon (9), and ammonium glufosinate + 2,4-D (10) were the treatments that caused the greatest damages (Figure 2A). The visual damage caused by these associations of herbicides can be evidenced by the higher concentration of ammonia at 2 and 4 DAA (Figure 2) when compared to the control and other treatments, which is possibly be related to a synergism that increased the absorption of ammonium glufosinate or both herbicides in the association.

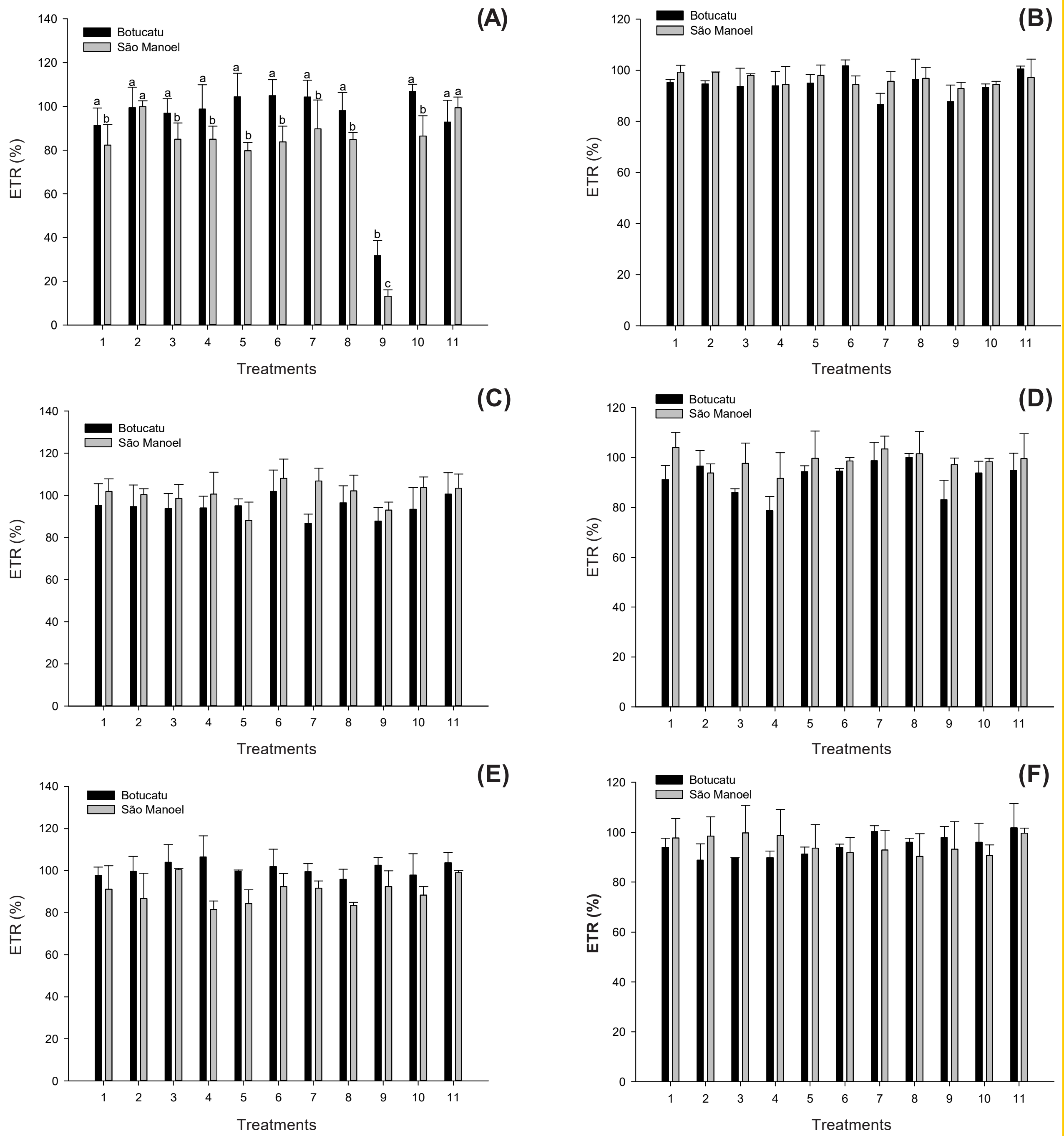
The associations of ammonium glufosinate + carfentrazone ethyl (8), ammonium glufosinate + bentazon (9), and ammonium glufosinate + 2,4-D (10) caused an increase in the concentration




Means followed by equal lowercase letters in columns of the same color do not differ significantly from each other by the Tukey's test ($p \leq 0.05$). 1 – ammonium glufosinate; 2 – glyphosate; 3 – ammonium glufosinate + glyphosate; 4 – ammonium glufosinate + nicosulfuron; 5 – ammonium glufosinate + atrazine; 6 – ammonium glufosinate + tembotrione; 7 – ammonium glufosinate + mesotrione; 8 – ammonium glufosinate + carfentrazone ethyl; 9 – ammonium glufosinate + bentazon; 10 – ammonium glufosinate + 2,4-D; 11 – control without weeding; and 12 – hand-weeded control.

***Figure 2 -*** Visual damage (%) of corn plants at 7 and 14 days (CV: 9.24 and 8.70%, respectively) and ammonia (treatments to control ratio) at 2 and 4 days after herbicide treatments application (CV: 13.19 and 19.33%, respectively) in post-emergence in the 2016/17 season in Botucatu and São Manoel, SP, Brazil. Means ± confidence interval.

of ammonia in relation to the control in the order of 28, 27, and 25 at 2 DAA and 9, 10, and 8 at 4 DAA, respectively (Figure 2). All treatments with ammonium glufosinate presented a higher ammonia accumulation when compared to the control without weeding (treatment to hand-weeded control ratio) and glyphosate application (2) (Figure 3) due to a GS inactivation after applying this herbicide, which stops ammonia metabolization (Manderscheid et al., 2005). Because




Means followed by equal lowercase letters in columns of the same color do not differ significantly from each other by the Tukey's test (p≤0.05). Graphs without letters were not significant. 1 – ammonium glufosinate; 2 – glyphosate; 3 – ammonium glufosinate + glyphosate; 4 – ammonium glufosinate + nicosulfuron; 5 – ammonium glufosinate + atrazine; 6 – ammonium glufosinate + tembotrione; 7 – ammonium glufosinate + mesotrione; 8 – ammonium glufosinate + carfentrazone ethyl; 9 – ammonium glufosinate + bentazon; 10 – ammonium glufosinate + 2,4-D; 11 – control without weeding; and 12 – hand-weeded control.

***Figure 3*** – ETR (%) in relation to the hand-weeded control at 3 h after application (A, CV: 7.89%) and at 1 (B, CV: 10.01%), 2 (C, CV: 8.32%), 3 (D, CV: 10.47%), 5 (E, CV: 6.10%), and 7 (F, CV: 4.17%) days after herbicide tratments application in the 2016/17 season in Botucatu and São Manoel, SP, Brazil. Means ± confidence interval.

the corn hybrid used shows the *pat* gene, the molecules of the herbicide ammonium glufosinate tend to be rapidly degraded into N-acetyl-L-glufosinate (Rojano-Delgado et al., 2013; Carbonari et al., 2016), guaranteeing GS activity and reducing plant intoxication by ammonia. Ammonia contents decreased at 4 DAA when compared to 2 DAA (Figure 2B). The reduction in ammonia contents a few days after ammonium glufosinate application was also observed in cotton, wheat, and sweet potato transformed with the *pat* gene (Shin et al., 2011; Rojano-Delgado et al., 2013; Carbonari et al., 2016).

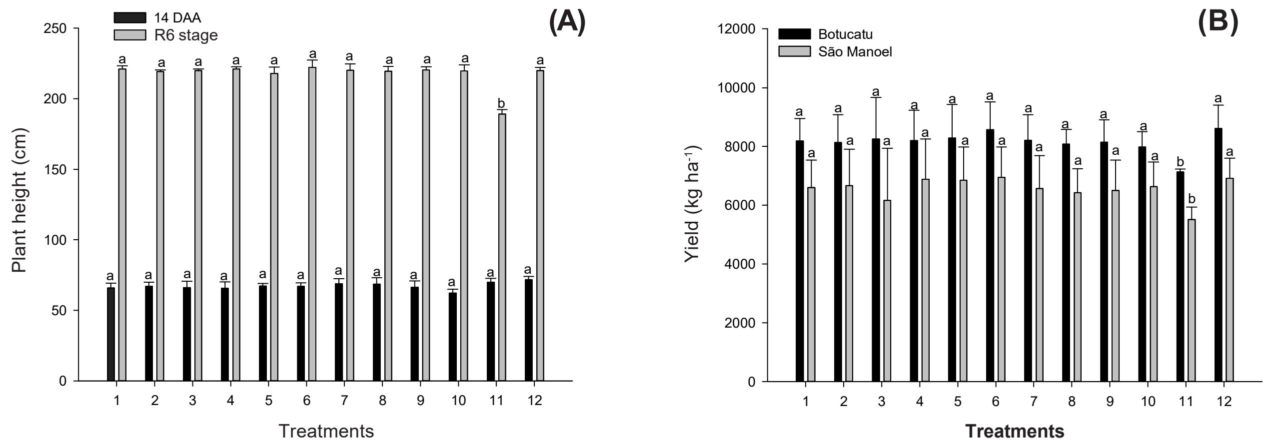
A secondary effect of ammonium glufosinate application is the reduced electron transport rate in photosynthesis (ETR). In the experiments conducted in Botucatu and São Manoel (Figure 3), ETR was significantly affected by the association of ammonium glufosinate + bentazon (9) 3 h after application, differing significantly from the other treatments. The herbicide bentazon inhibits the transport of electrons in photosystem II (Nimbal et al., 1996), leading to a rapid decrease in ETR, which is also reported in other species (Macedo et al., 2008; Araldi et al. 2015). A rapid recovery in ETR levels was observed in this association (ammonium glufosinate + bentazon) and, at 1 DAA, these levels were close to the other treatments (Figure 3B) since the herbicide bentazon did not cause a substantial damage to thylakoids (Macedo et al., 2008) and the PAT enzyme rapidly degraded the ammonium glufosinate.

In general, the percentage values of ETR for the experiment in Botucatu (Figure 3) were close to those of the hand-weeded control (100%). For the experiment conducted in São Manoel, significant differences were found 3 h after application and all treatments with ammonium glufosinate application caused a decrease of about 20% (except for ammonium glufosinate + bentazon (9)), when compared to the hand-weeded control and differed significantly from the control without weeding (11) and glyphosate (2) (Figure 3A). In the subsequent assessments (1, 2, 3, 5, and 7 DAA), no significant differences were observed between treatments nor values well below the hand-weeded control. Because the degradation of ammonium glufosinate by the PAT enzyme is rapid, with degradation metabolites being found in wheat 24 hours after application in plants with the *pat* gene (Rojano-Delgado et al., 2013) and 100% degradation in cotton with the *pat* gene (Carbonari et al., 2016), the recovery of ETR levels also occurs rapidly.

Herbicide application did not cause a reduction of corn plant height at 14 DAA. Thus, no significant difference was found between treatments (Figure 4). For plant height at the stage of physiological maturity (R6), only the control without weeding showed a significant reduction and differed from the other treatments (Figure 4A). Weed interference also caused a decrease in yield in both experimental areas (Figure 5), in which the control without weeding (11) differed significantly from the other treatments. When compared with the hand-weeded control, weed interference decreased yield by 17 and 20% for Botucatu and São Manoel. respectively. With the interference of *U. plantaginea*, corn showed a 62% lower yield in relation to treatments with of the control of this species (Galon et al., 2010). Dan et al. (2010) pointed out that weeds can cause losses of 13 to 88% in corn yield. The difference in productivity between sites may be explained by a lower soil fertility in the area of São Manoel (Table 1) since the climatic conditions of both sites are similar (Figure 1).

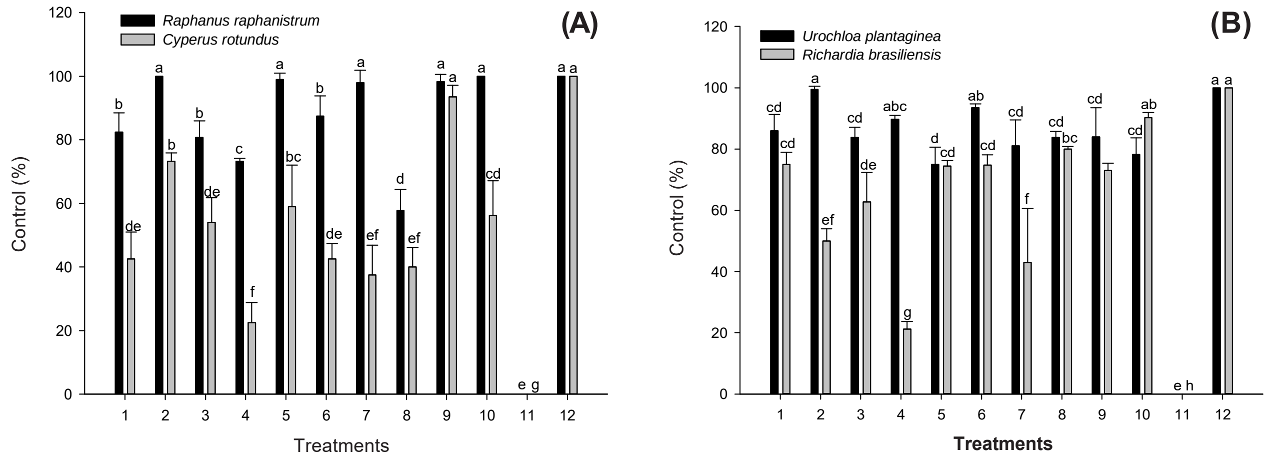
Ammonium glufosinate application and its associations did not promote a reduction in yield when compared to the hand-weeded control (Figure 4). Similarly, Silva et al. (2017) applied ammonium glufosinate alone and in association with glyphosate, atrazine, and the mixture of them and did not find a yield reduction in corn with the *cp4-epsps* and *pat* genes. Armel et al. (2008) assessed the mixture of ammonium glufosinate with mesotrione in corn with the *pat* gene and found no yield reduction with the interaction of both products and increases in doses. Despite some treatments have shown higher values of visual damages and a higher ammonia accumulation (Figure 2), they did not cause a yield reduction (Figure 4), which shows that corn has the capacity of recovering and keeping its yield.

The best herbicides for controlling *R. raphanistrum* (Figure 5) at 28 DAA were glyphosate (2), which provided a 100% control, and associations of ammonium glufosinate + atrazine (5), ammonium glufosinate + mesotrione (7), ammonium glufosinate + bentazon (9), and ammonium glufosinate + 2,4-D (10), which provided a 100% control. The other treatments showed satisfactory control, i.e. above 80%. The application of glyphosate, 2,4-D, and the association of both has been reported as having an efficient control of *R. raphanistrum* (Vitorino et al., 2014). For *C. rotundus*




Means ± confidence interval. Means followed by equal lowercase letters in columns of the same color do not differ significantly from each other by the Tobey test (p≤0.05). Graphs without letters were not significant. 1 – ammonium glufosinate; 2 – glyphosate; 3 – ammonium glufosinate + glyphosate; 4 – ammonium glufosinate + nicosulfuron; 5 – ammonium glufosinate + atrazine; 6 – ammonium glufosinate + tembotrione; 7 – ammonium glufosinate + mesotrione; 8 – ammonium glufosinate + carfentrazone ethyl; 9 – ammonium glufosinate + bentazon; 10 – ammonium glufosinate + 2,4-D; 11 – control without weeding; and 12 – hand-weeded control.

***Figure 4 -*** Corn plant height (cm) at 14 days after application (A, CV: 4.47%) and at the R6 stage (A, CV: 9.81%)] and corn yield (kg ha$^{-1}$) (B, CV: 7.83%) after herbicide treatments application in association in post-emergence in the 2016/17 in Botucatu and São Manoel, SP, Brazil.




Means followed by equal lowercase letters in columns of the same color do not differ significantly from each other by the Tukey's test (p≤0.05). 1 – ammonium glufosinate; 2 – glyphosate; 3 – ammonium glufosinate + glyphosate; 4 – ammonium glufosinate + nicosulfuron; 5 – ammonium glufosinate + atrazine; 6 – ammonium glufosinate + tembotrione; 7 – ammonium glufosinate + mesotrione; 8 – ammonium glufosinate + carfentrazone ethyl; 9 – ammonium glufosinate + bentazon; 10 – ammonium glufosinate + 2,4-D; 11 – control without weeding; and 12 – hand-weeded control.

***Figure 5 -*** Control (%) of the weed species *Raphanus raphanistrum*, *Cyperus rotundus* (A, CV: 4.40 and 13.50%, respectively), *Urochloa plantaginea*, *Richardia brasiliensis* (B, CV: 10.20 and 6.25, respectively) at 28 DAA in corn cultivation after herbicide treatments application in post-emergence in the 2016/17 season in Botucatu and São Manoel, SP, Brazil. Means ± confidence interval.

(Figure 5), the association of ammonium glufosinate + bentazon (9) provided a 95% control, not differing from the hand-weeded control, but differing from the other herbicides. Similar results for the control of *Cyperus* spp. were found with the herbicide bentazon in corn (Rezende et al., 2014).

The application of glyphosate (2) and ammonium glufosinate + tembotrione (6) promoted excellent control levels (above 95%), respectively, for *U. plantaginea* and did not differ significantly from the hand-weeded control (Figure 5B). However, the other treatments, with the exception of ammonium glufosinate + atrazine (5) and ammonium glufosinate + 2,4-D (10), caused a satisfactory control (above 80%). The species *U. plantaginea* is easily controlled with the application of ACCase inhibitor herbicides such as haloxyfop-methyl (Andrade Neto et al., 2016) and clethodim (Nonemacher et al., 2017), but the best option to control weeds in post-emergence in corn is the application of atrazine and nicosulfuron (Galon et al., 2010; Marchesan et al., 2013). Other management options are the application of glyphosate and ammonium glufosinate + tembotrione in corn resistant to these herbicides, which ensures the rotation of active principles in corn. For controlling *R. brasiliensis* (Figure 5), only the association of ammonium glufosinate + 2,4-D provided a 90% control, being statistically similar to the hand-weeded control. This species is considered a difficult-to-control plant (Takano et al., 2013) and its control has a higher efficiency with the association of herbicides. In fact, glyphosate + 2,4-D has provided a 100% control in plants with more than 10 leaves (Takano et al, 2013.).

The mixture of herbicides with ammonium glufosinate may be an alternative for weed control without causing damage to corn cultivars with the *cp4-epsps* and *pat* genes in its genetic constitution.

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