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# INSECT-RESISTANT MAIZE HYBRIDS TOLERANT TO GLUFOSINATE DUE TO THE *pat* GENE, UNDER DIFFERENT GLUFOSINATE RATES

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## ABSTRACT

**The use of glufosinate may have different effects on maize, with greater symptoms of injury for technologies with lower expression of the *pat* gene. As tolerance may vary with technology, it is important to investigate the selectivity of glufosinate in maize hybrids with the *pat* gene (resistant to insects). The objective of this study was to evaluate the selectivity of glufosinate rates applied in post-emergence, in insect-resistant transgenic maize with the *pat* gene. The experiment was carried out in Palotina, state of Paraná, Brazil, in 2020. A randomized block design with a factorial scheme (9x4) was used. The treatments were 9 maize hybrids and 4 glufosinate rates (0, 500, 700 and 1,000 g of active ingredient [ai] ha$^{-1}$). Injury symptoms, plant height, 1,000-grain mass and yield were evaluated. The results showed that injury increases with increasing glufosinate rates. For plant height and 1,000-grain mass, a significant effect was detected only for hybrids, while there was no effect on yield, even at the highest rates. Glufosinate was selective for insect-resistant maize hybrids with the *pat* gene, up to the rate of 1,000 g ai ha$^{-1}$, presenting initial injuries and some differences between maize hybrids. However, there was no impact on yield.**

**Key words:** glufosinate-tolerant maize, phosphinothricin N-acetyltransferase, glutamine synthetase, selectivity, agronomic performance.

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## INTRODUCTION

Glufosinate is a broad-spectrum, non-selective herbicide (selective only for crops with the *pat* and/or *bar* gene), derived from the natural toxin phosphinothricin, isolated from the fungi *Streptomyces viridochromogenes* and *Streptomyces hygroscopicus* (Dayan and Duke, 2014). It presents limited contact and translocation action. The first symptoms are the yellowing of leaves and other green tissues, followed by wilting and plant death. It acts by inhibiting the enzyme glutamine synthetase (GS), which is essential for nitrogen assimilation. With the inhibition of this enzyme, there is inhibition of the synthesis of amino acids and, consequently, of proteins (Takano et al., 2020; Takano and Dayan, 2020).

After uptake, plants show rapid accumulation of ammonia, accompanied by destruction of chloroplasts, reduced levels of photosynthesis, and decreased amino acid production (Barnett et al., 2012). The accumulation of ammonia in plant tissues due to GS inhibition is not enough to damage plant tissues, but GS inhibition rapidly increases the levels of reactive oxygen species, which are extremely phytotoxic, and cause loss of membrane integrity due to lipid peroxidation (Takano et al., 2019).

T25 and T14 transgenic events confer tolerance to the herbicide glufosinate in maize (Liberty Link™ - LL). Tolerance is conferred by the *pat* gene, originating from the *S. viridochromogenes* bacterium, which encodes the enzyme phosphinothricin N-acetyltransferase (PAT). This enzyme eliminates the herbicidal activity of glufosinate via acetylation (Matsuoka et al., 2001), while glufosinate is metabolized into N-acetyl-L-glufosinate (NAG), a non-toxic compound for plants (Müllner et al., 1993).

Bt11 (Agrisure™ CB/LL [TL]) and TC1507 (Herculex™ I [HX]) events, which are resistant to insects, also had the *pat* gene used as a marker in their selection process (Green et al., 2009). Therefore, hybrids with these insect resistance technologies, and other combinations with these events, are tolerant to glufosinate due to the *pat* gene in the selection process (Krenchinski et al., 2018; Nandula, 2019; Albrecht et al., 2021). In hybrids with these technologies, glufosinate has been used for post-emergent weed control. However, currently in Brazil, there are no commercially available hybrids of maize from T25 and T14 events - developed with the exclusive purpose of tolerance to glufosinate. Therefore, most scientific studies on the use of glufosinate in crops in Brazil are based on glufosinate-tolerant maize that is resistant to insects.

Araújo et al. (2021) observed the selectivity of glufosinate in insect-resistant maize, either for TC1507 x MON810 x MIR162 x NK603 (Leptra® RR2 [VYHR]) or MON89034 x TC1507 x NK603 (Power Core™ [PW]) events. This agrees with a previous study conducted by Krenchinski et al. (2019) on the effect of glufosinate applied in combination with other herbicides on PW maize. According to Krenchinski et al. (2018a), the expression of the *pat* gene is proportional to the level of tolerance to glufosinate. In insect-resistant hybrids, the expression of the *pat* gene was as follows: Leptra® (VYH) > PW > Optimum™ Intrasect (YHR) >> HX > TL = Agrisure® Viptera™ 3110 (VIP3). Therefore, the effects of glufosinate application on maize may vary, showing greater symptoms of injury for technologies with lower expression of the *pat* gene.

In this context, it is important to investigate the selectivity of glufosinate in maize hybrids with the *pat* gene (resistant to insects). Therefore, the aim of this study was to evaluate the selectivity of glufosinate rates applied in post-emergence, in insect-resistant transgenic maize plants with the *pat* gene.

## MATERIAL AND METHODS

The experiment was carried out in Palotina, state of Paraná (PR), Brazil (24°20'50"S 53°51'50"W) in 2020. The climate of the region is Cfa, according to the Köppen classification. The weather conditions for the experimental period are provided in Fig. 1.

Maize hybrids were sown under a no-till system on March 9, 2020. The soil at the experimental site had a very clayey texture, with a pH level of 4.8 ($CaCO_3$) and organic matter (OM) content of 2.23%. The experimental units consisted of 5-m long plots and 6 rows, with a row spacing of 0.45 cm. The experiment was set up in a randomized block design with four replications, in a factorial arrangement (hybrids x rates). Nine hybrids (Table 1) and 4 rates were used: 0; 500; 700 and 1,000 g of active ingredient (ai) $ha^{-1}$ glufosinate (Liberty®, 200 g ai $L^{-1}$, Basf S.A., Brazil). The maximum recommended rate of glufosinate for post-emergence applications in tolerant maize is 600 g ai $ha^{-1}$ (Rodrigues and Almeida, 2018). Applications of glufosinate above the maximum recommended rate are eventually used under field conditions. Therefore, it is necessary to investigate the selectivity of such rates.

The herbicide applications were conducted on March 30, 2020. The treatments were applied in post-emergence ($V_3$) of maize; 0.5 L $ha^{-1}$ adjuvant Mees® (Basf S.A., Brazil) was added to all glufosinate rates. A $CO_2$ pressurized backpack sprayer equipped with six AIXR 110.015 tips was

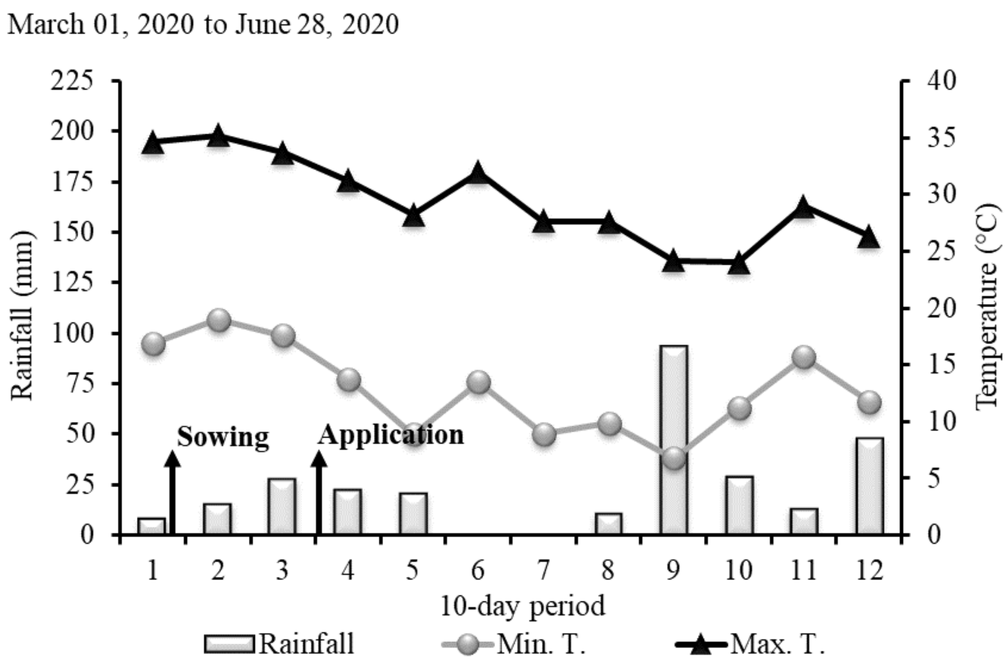


**Fig. 1. Rainfall and temperature during the experimental period. Palotina, PR, Brazil, 2020.**

**Table 1. Glufosinate-tolerant maize hybrids used in the experiment.**

| Hybrid | Technology | Event |
|---|---|---|
| FS575 | Power Core (PW) | MON89034 x **TC1507** x NK603 |
| FS403 | Power Core (PW) | MON89034 x **TC1507** x NK603 |
| FS533 | Power Core Ultra (PWU) | MON89034 x **TC1507** x NK603 x MIR162 |
| FS500 | Power Core Ultra (PWU) | MON89034 x **TC1507** x NK603 x MIR162 |
| SYN488 | Agrisure Viptera 3110 (VIP3) | **Bt11** x MIR162 x GA21 |
| SX7341 | Agrisure Viptera 3110 (VIP3) | **Bt11** x MIR162 x GA21 |
| SYN422 | Agrisure Viptera 3110 (VIP3) | **Bt11** x MIR162 x GA21 |
| MG408 | Power Core Ultra (PWU) | MON89034 x **TC1507** x NK603 x MIR162 |
| P30F53 | Leptra RR2 (VYHR) | **TC1507** x MON810 x MIR162 x NK603 |

[1]Events confer tolerance to glufosinate due to insertion of the *pat* gene

used, at a pressure of 2 kgf $cm^{-2}$ and a speed of 3.6 km $h^{-1}$, providing an application volume of 150 L $ha^{-1}$. The herbicide was applied at a temperature of 31.2 ºC, relative humidity of 52% and no wind.

Injury symptoms were evaluated in maize plants at 7, 14, 21 and 28 days after application (DAA). For all of these, scores were assigned through visual analysis to each experimental unit (0 for no injuries, up to 100% for plant death). Based on the significantly visible symptoms in the plants according to their development (Velini et al., 1995), the main symptoms that plants show after the application of glufosinate are chlorosis, followed by necrosis (Barnett et al., 2012; Albrecht et al., 2020)

At pre-harvest, eight maize plants were randomly selected per plot, and plant height was measured as the distance between the soil surface and the base of the inflorescence. To determine yield, 4 rows were harvested from each plot (4-m long). The grains produced in each plot had their mass measured and moisture corrected to 13%. From these data, yield in kg $ha^{-1}$ was calculated. For each plot, the mass of 8 sub-samples of 100 grains each was also measured. The average value was multiplied by ten, moisture was corrected to 13%, and the mass of 1,000 grains was determined.

Data were subjected to analysis of variance (ANOVA) by the F-test ($p < 0.05$) according to Pimentel-Gomes and Garcia (2002). The basic assumptions for ANOVA were met and all necessary analyses were carried out. For maize hybrids, mean values per treatment were grouped using the Scott and Knott (1974) test ($p < 0.05$). For rates, mean values were subjected to regression analysis ($p < 0.05$). To select the best regression model, the following fit criteria were followed: biological explanation, significant regression, non-significant regression deviations and coefficient of determination. For this purpose, the Sisvar 5.6 software was used (Ferreira, 2011). Microsoft 365® Excel® (Microsoft Corp.) software was used to create the figures.

## RESULTS AND DISCUSSION

The ANOVA results showed a significant effect ($p < 0.05$) of glufosinate on injury symptoms at 7 and 14 DAA for hybrids, rates and factor interaction. The same behavior was observed at 21 DAA for hybrids and rates, but not for interaction. For plant height and 1,000-grain mass, there was a significant effect only for hybrids, while no effect was detected in terms of yield (Table 2).

Data on injury symptoms fit an increasing linear model, with an increase in injuries with increasing rates (Figs. 2-4), even when the injury values were very low, and the plants fully recovered after 28 DAA. Up to 14 DAA, there was interaction between the factors. In general, injury was higher for the PW and SYN488 VIP3 hybrids. At 21 DAA, no interaction with symptoms was observed in the average of rates for the hybrids of at most 0.4% (Table 3). At 28 DAA, no further injury symptoms were observed in all the hybrids.

For height and 1,000-grain mass, differences were only found in hybrids, while rates had no effects on these variables. In addition, no differences were found in yield, which reinforces the selectivity of glufosinate rates regardless of the hybrid (Table 4).

The selectivity of glufosinate, alone or in combinations, has been described in previous studies in maize (Legleiter and Bradley, 2009; Lindsey et al., 2012; Ganie and Jhala, 2017). In these studies, the maize hybrids were T14 or T25 events, that is, the LL technology (properly said) guarantees a good level of selectivity to the maize plants.

At present, a greater potential for glufosinate injury was observed in hybrids FS575 PW and FS403 PW, SYN488 VIP3. Nevertheless, symptoms in absolute values were 6.3% at 7 DAA, without injuries at 28 DAA; no differences in yield and other variables related to agronomic performance were found. Less potential for injury was observed in the PWU or VYHR hybrids. These differences might be ascribed to the different expression levels of the *pat* gene in the technologies (Krenchinski et al., 2018a). However, differences were also observed between hybrids of the same technology, e.g., VIP3. This means that characteristics of each hybrid in addition to the transgenic event can interfere with tolerance to glufosinate.

In addition, glufosinate selectivity for insect-resistant hybrids has been observed at a rate of 500 g ai ha$^{-1}$ for the PW technology (Silva et al., 2017a; Krenchinski et al., 2018b; Krenchinski et al., 2019; Krenchinski et al., 2020a) or YHR at the rate of 600 g ai ha$^{-1}$ (Costa et al., 2018). Similarly, Krenchinski et al. (2020b) determined the selectivity of glufosinate in maize with technologies VIP3, HX, TL, YHR, VYH and PW, with greater injury especially in TL hybrid, but no reductions in yield.

Araújo et al. (2021) observed the selectivity of glufosinate in insect-resistant maize, VYHR or PW. Rates of 1,500, 3,000, 4,500 and 6,000 g ai ha$^{-1}$ were applied, with injury symptoms of more than 30% in some evaluations. Despite the injury level, no deleterious effects were observed on the nutritional content or yield of maize. The maximum recommended rate of glufosinate for

**Table 2. Results (F-test) of the ANOVA.**

| Source | Injury 7 DAA | Injury 14 DAA | Injury 21 DAA | Height | 1,000-grain mass | Yield |
|---|---|---|---|---|---|---|
| | | % | | cm | g | kg ha$^{-1}$ |
| Hybrid (H) | 11.9* | 5.8* | 2.3* | 6.5* | 9.4* | 1.5$^{ns}$ |
| Rates (R) | 120.6* | 73.8* | 21.8* | 0.3$^{ns}$ | 1.0$^{ns}$ | 0.1$^{ns}$ |
| H x R | 3.0* | 3.3* | 1.2$^{ns}$ | 0.3$^{ns}$ | 0.6$^{ns}$ | 0.1$^{ns}$ |
| Means | 1.6 | 0.8 | 0.2 | 190.3 | 450.7 | 8,006 |
| CV (%) | 16.8 | 17.6 | 13.8 | 5.5 | 6.2 | 14.2 |

*Significant (p < 0.05), means differ by the F-test.

$^{ns}$ Non-significant (p > 0.05), means do not differ by the F-test.

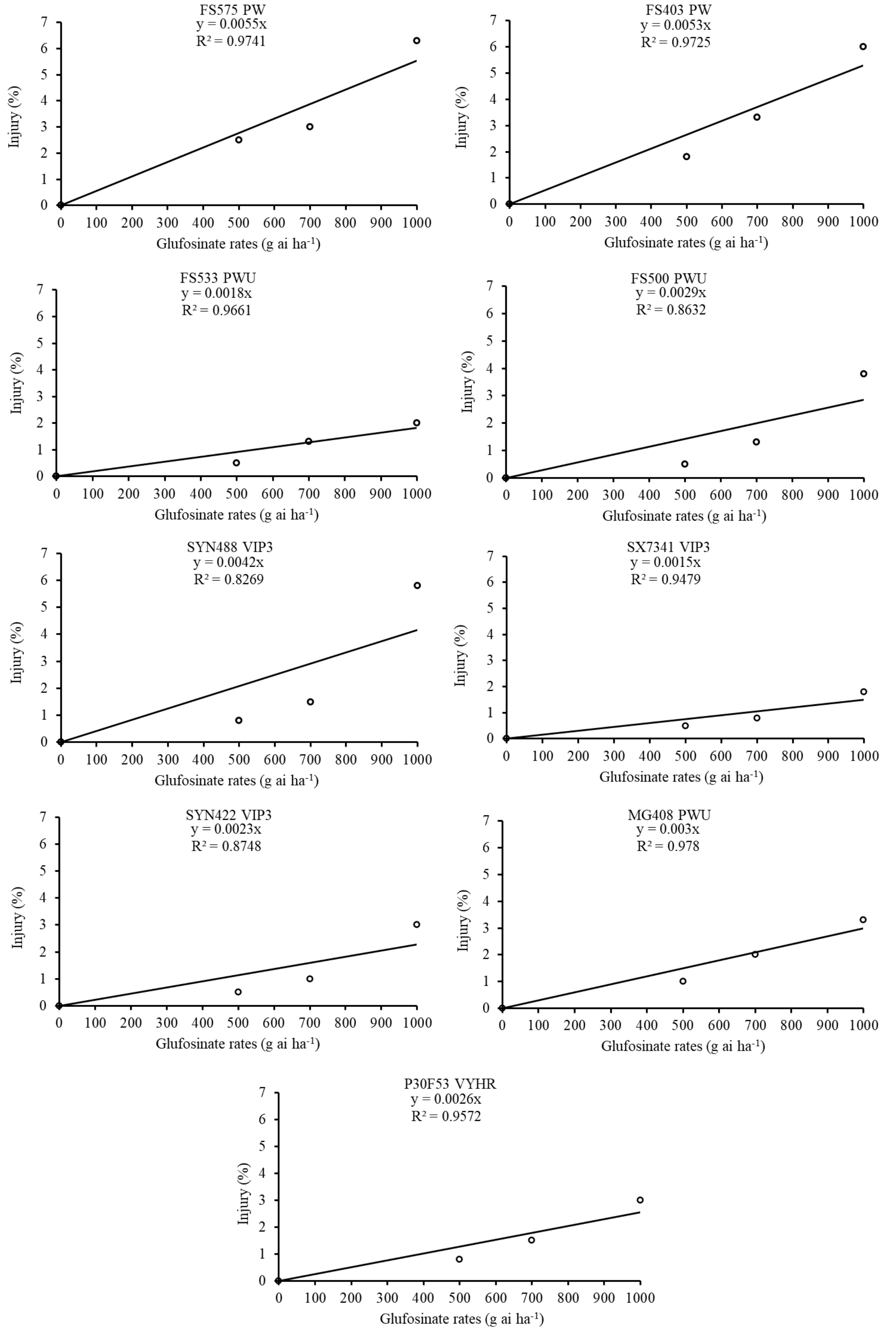


**Fig. 2. Injury in maize hybrids at 7 DAA of glufosinate rates.**

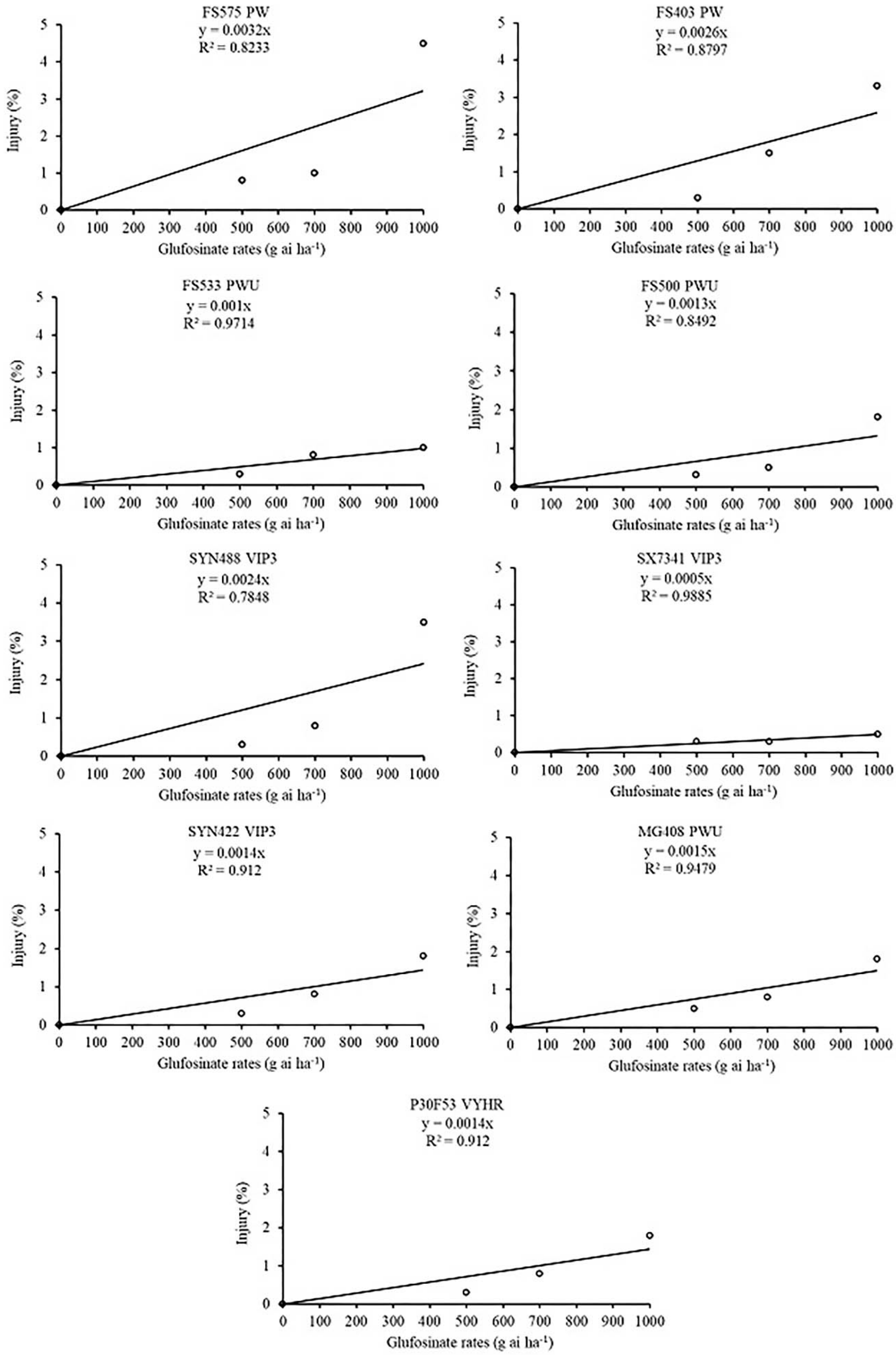


**Fig. 3. Injury in maize hybrids at 14 DAA of glufosinate rates.**

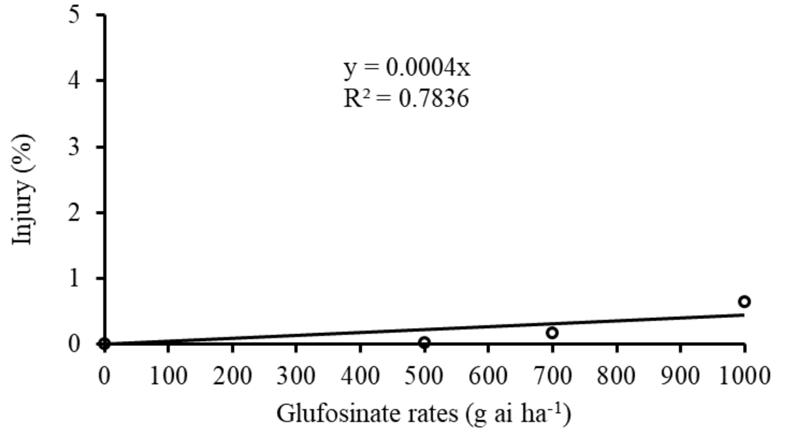


**Fig. 4. Injury in maize hybrids (mean of hybrids) at 21 DAA of glufosinate rates.**

**Table 3. Injury in maize hybrids at 7, 14 and 21 DAA of glufosinate rates.**

| | 7 DAA | | | | 14 DAA | | | | 21 DAA |
|---|---|---|---|---|---|---|---|---|---|
| | Rate | | | | | | | | |
| **Hybrid** | 0 | 500 | 700 | 1,000 | 0 | 500 | 700 | 1,000 | Mean |
| | % | | | | | | | | |
| FS575 PW | 0 a | 2.5 b | 3.0 b | 6.3 c | 0 a | 0.8 a | 1.0 a | 4.5 d | 0.4 b |
| FS403 PW | 0 a | 1.8 b | 3.3 b | 6.0 c | 0 a | 0.3 a | 1.5 a | 3.3 c | 0.4 b |
| FS533 PWU | 0 a | 0.5 a | 1.3 a | 2.0 a | 0 a | 0.3 a | 0.8 a | 1.0 a | 0.1 a |
| FS500 PWU | 0 a | 0.5 a | 1.3 a | 3.8 b | 0 a | 0.3 a | 0.5 a | 1.8 b | 0.1 a |
| SYN488 VIP3 | 0 a | 0.8 a | 1.5 a | 5.8 c | 0 a | 0.3 a | 0.8 a | 3.5 c | 0.3 b |
| SX7341 VIP3 | 0 a | 0.5 a | 0.8 a | 1.8 a | 0 a | 0.3 a | 0.3 a | 0.5 a | 0.1 a |
| SYN422 VIP3 | 0 a | 0.5 a | 1.0 a | 3.0 b | 0 a | 0.3 a | 0.8 a | 1.8 b | 0.1 a |
| MG408 PWU | 0 a | 1.0 a | 2.0 a | 3.3 b | 0 a | 0.5 a | 0.8 a | 1.8 b | 0.2 a |
| P30F53 VYHR | 0 a | 0.8 a | 1.5 a | 3.0 b | 0 a | 0.3 a | 0.8 a | 1.8 b | 0.3 b |

Means followed by the same letter in the rows (comparison between hybrids) do not differ by the Scott and Knott (1974) test, at the 5% significance level.

**Table 4. Plant height, 1,000-grains mass, and yield of hybrid maize under the application of glufosinate rates.**

| **Hybrid** | **Height*** | **1,000-grain mass*** | **Yield$^{ns}$** |
|---|---|---|---|
| | cm | g | kg ha$^{-1}$ |
| FS575 PW | 197.6 a | 459.5 a | 8,231 |
| FS403 PW | 195.1 a | 464.5 a | 7,865 |
| FS533 PWU | 194.9 a | 456.3 a | 7,999 |
| FS500 PWU | 182.1 c | 463.9 a | 7,527 |
| SYN488 VIP3 | 188.2 b | 409.3 c | 7,776 |
| SX7341 VIP3 | 188.4 b | 474.6 a | 8,388 |
| SYN422 VIP3 | 188.9 b | 436.3 b | 7,778 |
| MG408 PWU | 189.9 b | 463.9 a | 8,405 |
| P30F53 VYHR | 179.2 c | 428.2 b | 7,552 |

*Means followed by the same letter in the rows (comparison between hybrids) do not differ by the Scott and Knott (1974) test, at the 5% significance level.
$^{ns}$ Non-significant ($p > 0.05$), means do not differ by the F-test.

post-emergence applications in tolerant maize is 600 g ai ha$^{-1}$ (Rodrigues and Almeida, 2018). In the present study, injuries were observed at the highest rates, with no deleterious effects on maize yield.

Even though there was no impact on yield, rates above the package insert have a greater potential for injury. Studies evaluating the effect of glufosinate application above the recommended rate in LL maize (T25 and T14 events) are scarce. The studies cited here, with high rates of glufosinate, were carried out with insect-resistant hybrids. In glufosinate-tolerant soybean, in which the *pat* gene was not used as a marker, glufosinate selectivity was observed, with symptoms of injury for application above the recommended rate, but yield was not affected (Albrecht et al., 2020; Mundt et al., 2021).

In recent years, in Brazil, tolerance to glufosinate has been increasingly used for post-emergent weed management in insect-resistant maize. Bt11 and TC1507 events provide good levels of tolerance to glufosinate - up to a rate of 1,000 g ai ha$^{-1}$ - as it was demonstrated in the present study. In general, the injury caused by herbicides in transgenic crops occurs when applications are made above the recommended rate, and/or outside a specific growth stage. Therefore, glufosinate can be regarded as a safe herbicide for maize plants with the *pat* gene (marker).

In this context, glufosinate can be an interesting tool for weed management in maize, particularly for controlling weeds of the family Poaceae since there are few effective options for post-emergence application in maize. The application of glufosinate was effective in controlling *Digitaria* spp. (Craigmyle et al., 2013; Silva et al., 2017b; Randel et al., 2020), which highlights the potential of this herbicide for weed management in maize.

## CONCLUSION

Glufosinate was selective for insect-resistant maize hybrids, with the *pat* gene, up to the maximum applied rate (1,000 g ai ha$^{-1}$), with initial injuries for the highest rates and some differences between the hybrids. However, there was no impact on yield.

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