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Digitaria insularis: cross-resistance between ACCase inhibitors and multiple resistance to glyphosate

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Abstract

Sourgrass (*Digitaria insularis* [L.] Mez ex Ekman) is a problematic weed with special attention to its damage and herbicide resistance in Brazil and Paraguay. Considering the complexity of management and failure in control approaches, the goal of the present study was to monitor and investigate herbicide resistance in *D. insularis.* Furthermore, we specifically analyze a case with multiple resistance in Paraguay. In this sense, studies were carried out for the development of dose-response curves. The results showed resistance of *D. insularis* to ACCase- and EPSPs-inhibiting herbicides in Paraguay, in which the resistance to ACCase inhibitors involves the chemical groups aryloxyphenoxypropionates and cyclohexanediones. This is probably the first case of resistance observed in the world. It is necessary to apply good practices in the set of integrated weed management. These practices should be accessed as quickly and effectively as possible, serving as a lesson for more proactive and less reactive actions in production systems.

Keywords: Sourgrass, Haloxyfop, Clethodim, EPSPs inhibitors, Herbicide resistance.
Abbreviations: AMPA_aminomethylphosphonic acid, C_{50}_control by 50%, C_{80}control by 80%, DAA_days after application, DIM_cyclohexanediones, FOP_aryloxyphenoxypropionate, GR_{50}_growth reduction by 50%, GR_{80}_growth reduction by 80%, RF_resistance factor.

Introduction

Sourgrass (*Digitaria insularis* [L.] Mez ex Ekman) is a plant native to tropical and subtropical regions of America (Veldman and Putz, 2011), occurring in Brazil and Paraguay (Lorenzi, 2014; Moreira and Bragança, 2010). This is a species with high aggressiveness, which occupies the most diverse production systems, presenting underground rhizome and aerial, cylindrical and canaliculated stem, little or no branching, and with great adaptive capacity and propagule production (Machado et al., 2008; Moreira and Bragança, 2010; Gemelli et al., 2012; Lorenzi, 2014).
With the introduction of the no-till system, this species scattered rapidly due to its aggressive characteristics (Gemelli et al., 2013), making it difficult to control. The coexistence of 8 Sourgrass plants m^2 with the soybean crop is enough to reduce yield by about 80% (Gazziero et al., 2019; Braz et al., 2021). In recent years, potential losses associated with the repeated use of herbicides with the same mechanism of action has caused selection pressure, with the consequent emergence of resistant populations.
The first case of *D. insularis* resistance to the herbicide glyphosate was registered in Paraguay in 2005. In Brazil, sourgrass resistance to glyphosate was recorded for the first time in 2008, in the state of Paraná (Heap, 2022). In 2009, a case of resistance to glyphosate was identified in the state of São Paulo (Carvalho et al., 2011). Takano et al. (2018) analysed the resistant biotypes in Paraguay and the states of Paraná and São Paulo. They concluded that populations of *D. insularis* from Paraguay and Paraná share a similar genetic basis. So, it is possible that resistance from Paraguay has spread, and will spread further, to Paraná through gene flow.
In 2016, in the Midwest region of the country (Brazil), resistance of *D. insularis* to ACCase-inhibiting herbicides, such as fenoxaprop and haloxyfop was recorded (Heap, 2022). More recently, a resistant biotype was reported in the state of Mato Grosso, resistant to haloxyfop and pinoxaden (Takano et al., 2020), also a biotype with multiple resistance to glyphosate and ACCase inhibitors (haloxyfop and fenoxaprop) (Heap, 2022).
Regarding the resistance mechanisms to glyphosate, Carvalho et al. (2012) observed that the herbicide was degraded (>90%) to AMPA, glyoxylate and sarcosine in the resistant biotypes, while a small amount of herbicide (up to 11%) was degraded by the susceptible biotype at 168 hours after the treatment. In addition, two amino acid changes were found at positions 182 and 310 in the sequencing of the gene encoding EPSPs in resistant biotypes. The differential metabolism of glyphosate, with rapid degradation of the herbicide in the resistant biotype was again identified as a resistance mechanism in *D. insularis* (Carvalho et al., 2013). Other studies have evaluated herbicide translocation (Melo et al., 2019), differential metabolism (Gazola et al., 2020), the importance of

compounds such as salicylic acid (Gaille et al., 2002), the impact of leaf anatomy and absorption (Barroso et al., 2015). Therefore, uptake, translocation, metabolism and gene mutation may play an important role in glyphosate resistance in *D. insularis*.

In the case of resistance of *D. insularis* to haloxyfop and pinoxaden, a mutation at the site of action, Trp2027Cys, was found in resistant plants, which prevents the access of FOP herbicides to the site. This mutation does not cause resistance to DIM (Takano et al., 2020). However, this mutation has not yet been identified as responsible in other *D. insularis* biotypes resistant to ACCase-inhibiting herbicides.

Considering the whole problem, it is essential to work more with integrated weed management, involving a large set of good practices. This includes strategies that range from monitoring, through phytosociological survey and identification of resistance, and that result in agricultural control practices, such as cultural, mechanical and chemical practices (Drehmer et al., 2015; Silva et al., 2017; Marochi et al., 2018; Raimondi et al., 2019; Albrecht et al., 2020a).

Since 2016, in order to characterize the challenges present in Paraguay, studies have been conducted to build alternatives by the Federal University of Paraná (Brazil) in partnership with Paraguay. The studies mainly focus on unraveling the problem of weed resistance to herbicides and generating solutions. Nevertheless, much still needs to be investigated and built together, as there are many problems in weed management that Brazil and Paraguay share. Furthermore, problems raised in this region can be dispersed throughout Latin America and serve as a study model to configure actions in other parts of the planet.

This study was carried out with the objective of monitoring and investigating the resistance of *D. insularis* to herbicides, specifically to analyze a case with an indication of multiple resistance. The work was conducted in conjunction with researchers, technicians and farmers from Paraguay, a country on a common border with Brazil, which presents similar challenges, but with a great lack of in-depth research on the science of weeds.

Results and Discussion

Dose-response

After statistical analysis and application of non-linear regression, models and graphs were generated as commonly adopted (Burgos et al., 2013; Takano et al., 2016; Takano et al., 2017; Zobiole et al., 2019; Albrecht et al. 2020b; Albrecht et al., 2020c). Figures 1 and 2 show the control of dry mass at 28 days after glyphosate application; while Figures 3 and 4 refer to haloxyfop; Figures 5 and 6, to clethodim. C_{50}, GR_{50} and RF values are shown in the Figures. All RF were greater than 4, indicating resistance of the biotype compared to the susceptible.

The C_{80} was also calculated for resistant biotypes (noting that C_{50} and GR_{50} are in the figures), obtaining the following values: glyphosate = 122,232.61 (87.31 times higher than the label dose); haloxyfop = 1499.60 (8.24 times higher than the label dose); clethodim = 525.09 (2.73 times higher than the label dose). These high values clearly show the multiple resistance of the evaluated biotype, even when compared to the literature (Carvalho et al., 2011; Takano et al., 2017; Takano et al., 2018; Takano et al., 2020).

Confirmation of the resistance case

This confirms the resistance of *D. insularis* to ACCase- (group A) and EPSP- (group G) inhibiting herbicides. Resistance to ACCase-inhibiting herbicides involves the chemical groups FOP and DIM. Therefore, it is a cross-resistance within the mechanism of action and multiple between the mechanisms, representing the first case in the world with this particularity for the species in question. Nevertheless, the resistance mechanism still needs to be elucidated. In this sense, other partners will be helping in the next phases of the research.

Resistance of *D. insularis* to graminicide herbicides and glyphosate may reveal distinct and combined resistance mechanisms. The complexity of the topic and the need for exhaustive studies can be evidenced by the relevant literature (Gaille et al., 2002; Carvalho et al.; 2012; Carvalho et al., 2013; Barroso et al., 2015; Melo et al., 2019; Gazola et al., 2020; Takano et al., 2020).

Regarding the communication of resistance, the *D. insularis* population meets all established criteria to confirm a new case of multiple resistance to glyphosate, haloxyfop and clethodim (Heap, 2005). Criterion 1: plants from these populations survived and reproduced, after exposure to a herbicide dose lethal to the susceptible population; Criterion 2: resistance factors were high and the recommended dose for the species did not provide satisfactory control; Criterion 3: plants of the F1/F2 generation of these populations were considered resistant; Criterion 4: complaints of control failures have been observed in the field; Criterion 5: random plants from this population were properly classified as *D. insularis*. This culminated in their report (Heap, 2022).

The Brazilian Society of Weed Sciences was notified about this case of multiple resistance and following the HRAC-Brasil. Even being a case found in Paraguay, this problem with the target species affects both countries and there is a large agricultural border region potentially affected by this challenge.

Implications and management

At this moment, monitoring actions are being carried out in the areas where resistant biotypes were found, as well as in other areas with suspected resistance. This work is being carried out in partnership with FARM Consultoria & Investigación Agronomica and with the collaboration of farmers and technicians from different institutions operating in Paraguay. The main focus is on alerting farmers about this problem and reducing its spread in Paraguay and Brazil to avoid the loss of these important tools.

Importantly, since June 2021, these results have been released and discussed with professionals and farmers in Paraguay and Brazil. This was done in person and through different social media. In this sense, technical reports were prepared and disseminated by the authors of this study to inform and raise awareness in people affected by this drama in Brazil and Paraguay. Results by Takano et al. (2018), Takano et al. (2020), Albrecht et al. (2020b), and Albrecht et al. (2020c) pointed the importance of continuing studies on monitoring and mapping resistance.

In addition to preventive practices, after diagnosing *D. insularis* herbicide resistance, several remedial actions can

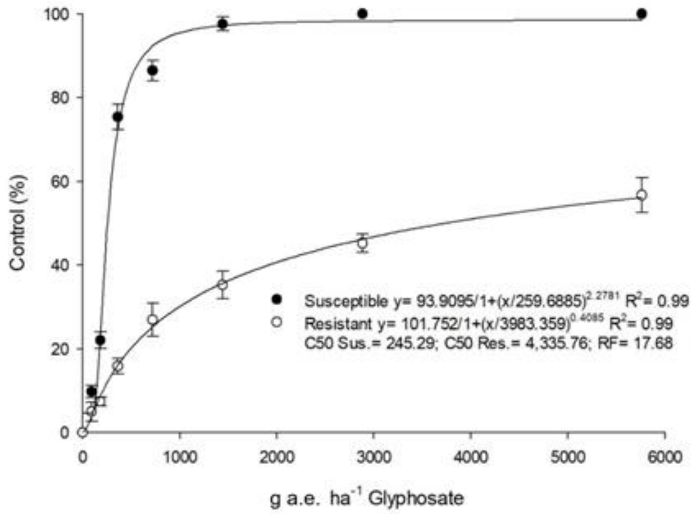

Figure 1. Control (%) of *D. insularis* at 28 days after glyphosate application. District of Hernandarias, Department of Alto Paraná - Paraguay, 2021.

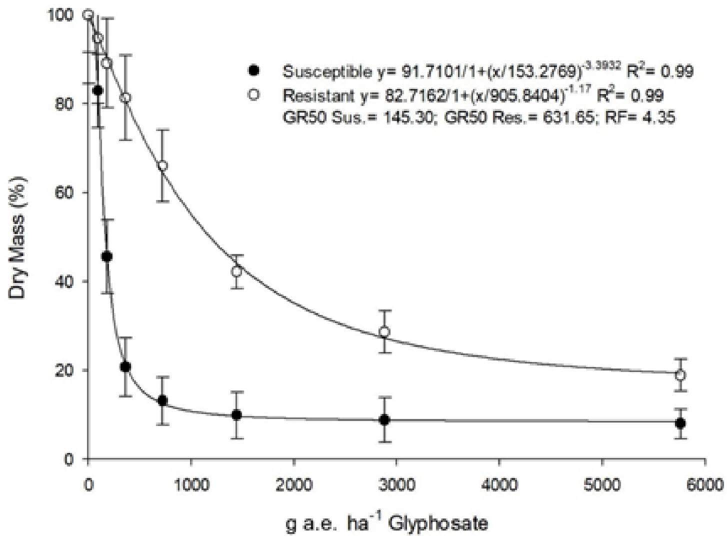

Figure 2. Dry mass (%) of *D. insularis* at 28 days after glyphosate application. District of Hernandarias, Department of Alto Paraná - Paraguay, 2021.

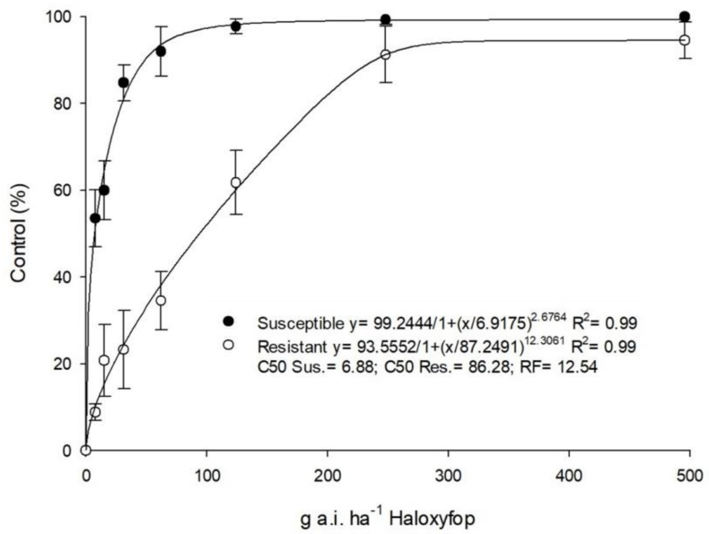

Figure 3. Control (%) of *D. insularis* at 28 days after haloxyfop application. District of Hernandarias, Department of Alto Paraná - Paraguay, 2021.

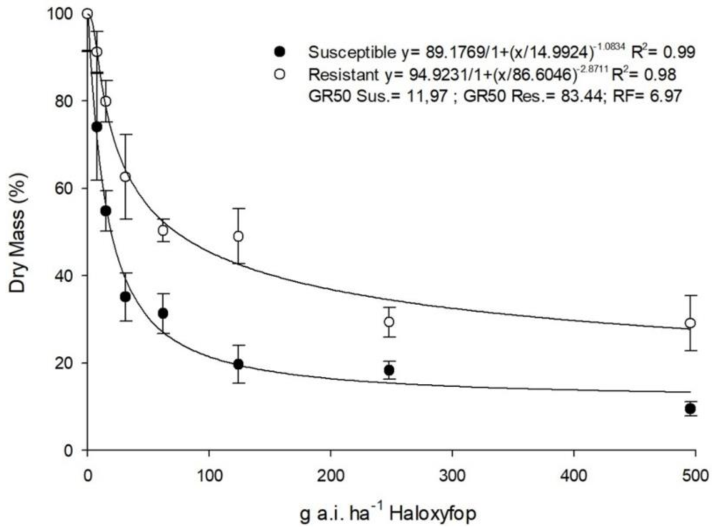

Figure 4. Dry mass (%) of *D. insularis* at 28 days after haloxyfop application. District of Hernandarias, Department of Alto Paraná - Paraguay, 2021.

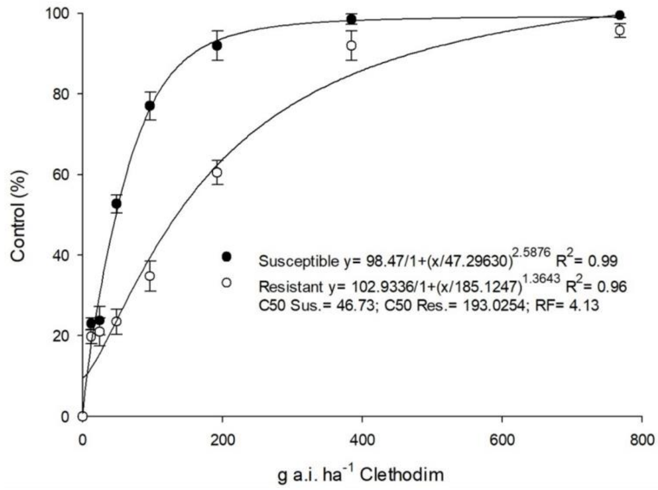

Figure 5. Control (%) of *D. insularis* at 28 days after clethodim application. District of Hernandarias, Department of Alto Paraná - Paraguay, 2021.

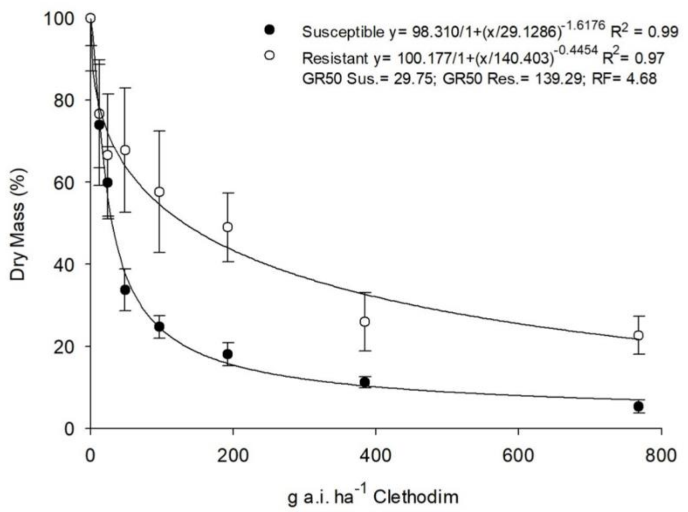

Figure 6. Dry mass (%) of *D. insularis* at 28 days after clethodim application. District of Hernandarias, Department of Alto Paraná - Paraguay, 2021.

be taken, such as chemical control, within the logic of integrated weed management. There are several practices for the management of glyphosate-resistant *D. insularis*, which are: burndown at the initial development stage to prevent the controlled plants from producing seeds, rotation of mechanisms of action and chemical groups, besides other agricultural practices such as cleaning of the machines and harvesters after harvesting, weeding, crop rotation, mowing, cover crops. Also, in relation to desiccation, it must be done well in advance of planting and when necessary, complement the application with other products (Oliveira Júnior et al., 2006; Canedo et al., 2019).

As for the use of herbicides, to control glyphosate-resistant *D. insularis*, a widely used alternative is the use of ACCase-inhibiting herbicides, such as clethodim and haloxyfop, in complementary or synergistic combinations (Barroso et al., 2014; Cassol et al., 2019; Gilo et al., 2016; Bianchi et al., 2020; Bauer et al., 2021; Onofre et al., 2021). These herbicides are generally effective in early stages of development (Presoto et al., 2020). Nevertheless, it is important to note that as the plant has the ability to re-sprout, the single application of herbicides, even at high doses, is not sufficient for an effective control of perennial plants, requiring sequential applications (Cassol et al. al., 2019; Zobiole et al., 2016; Mendes et al., 2020). However, the report of resistance in Paraguay potentially hampers the implementation of such control practices in an area with proven presence of problem-populations.

The use of pre-emergence herbicides such as s-metolachlor, flumioxazin, imazethapyr, sulfentrazone, clomazone, diclosulam, pyroxasulfone, could help better management of resistant *D. insularis* (Drehmer et al., 2015), with effective control in management systems with cover crops (Marochi et al., 2018). Mixtures of imazapic and imazapyr (Albrecht et al., 2020a) or glufosinate (Silva et al., 2017) can be applied in the off-season to control resistant *D. insularis*. The combination of chemical control with mowing is also effective in controlling *D. insularis*, being an alternative especially for perennial plants (Raimondi et al., 2019; Raimondi et al., 2020).

With the discovery of multiple resistance and the complications in management, the urgent need to apply good practices in the set of integrated weed management is evident. These practices are accessed as quickly and effectively as possible, serving as a lesson for more proactive and less reactive actions in production systems. This may serve as a global alert, in taking care of the increase in cases of weed resistance to herbicides or a global pandemic.

Materials and Methods

Monitoring of Digitaria insularis

The Supra Pesquisa team, from the Federal University of Paraná, carries out joint activities with partners in Paraguay. As of 2017, monitoring initiatives started with FARM Consultoria and Investigación Agronomica, which led to the mapping of escape areas or areas with control failures. Indicators of *D. insularis* resistance to herbicides were monitored and evaluated.

Seeds of *D. insularis* were collected during the 2019/2020 growing season. In the first half of 2020, screening was performed to select biotypes to be used for dose response curves as susceptible and resistant. Plants of these biotypes were cultivated and their seeds collected for the development of dose response curves, which were carried out in the second half of 2020 in a greenhouse, located in the District of Hernandarias, Department of Alto Paraná, Paraguay.

Between June and December 2020, field experiments were conducted in the area that presented the resistant biotype in dose response curves, located in the District of Hernandarias, Department of Alto Paraná, Paraguay (25°20'54.2"S 54°40'58.3"W). These experiments were undertaken for practical field evaluation of *D. insularis* resistant to three herbicides studied and to find efficient control alternatives in areas with this problem. Plants at the reproductive stage were properly identified as *D. insularis*.

In the focus area of the samples with indication of resistance, the target species was later eradicated, and good practices were contingently carried out, avoiding the propagation of the potential problem. *Digitaria insularis* plants were cultivated under controlled conditions and heritability was conducted, following internationally adopted criteria (Heap, 2005; Burgos et al., 2013).

Dose-response experiment

After screening, seeds of the F_1 generation (first generation) were sown and after emergence, thinned, leaving one seedling per pot with six repetitions. Herbicides tested in F_1 were glyphosate (720 g a.e. L^{-1}), haloxyfop (62 g a.i. L^{-1}), and clethodim (96 g a.i. kg^{-1}).

The experiment in the F_2 generation (second generation) was conducted in a completely randomized design with four replications. The treatments consisted of: glyphosate at doses of 0, 90, 180, 360, 720, 1440, 2,880 and 5760 (g a.e. ha^{-1}); haloxyfop at doses of 0, 7.5, 15, 31, 62, 124, 248 and 496 (g a.i. ha^{-1}) combined with 0.5% (v/v) emulsifiable mineral oil; and clethodim at 0, 12, 24, 48, 96, 192, 384 and 768 (g a.i. ha^{-1}) combined with 0.5% (v/v) emulsifiable mineral oil. The doses used represent the normal field doses at 0, 1/8, ¼, ½, 1, 2, 4 and 8X doses.

Experimental units were pots containing 1.0 dm^{-3} vermiculite, under greenhouse conditions. Treatments were applied to plants with three tillers, one plant per pot. All herbicide applications were made using a CO_2 pressurized backpack sprayer equipped with four flat-fan nozzles AIXR-110015 (TeeJet Technologies, Wheaton, IL) at a pressure of 240 kPa and a speed of 1 ms^{-1}, delivering an application volume equivalent to 200 L ha^{-1}.

Control was evaluated at 7, 14, 21 and 28 DAA of the herbicides, through visual evaluation (0 for no injuries, up to 100% for plant death). In this case, symptoms significantly visible in plants, according to their development (Velini et al., 1995). Dry mass was evaluated at 28 days after herbicide application. Plants were cut at the soil surface, stored in paper bags, oven dried at 70 °C for 4 days (to constant mass) and then weighed. Data were tested by analysis of variance and regression, and when significant, were fitted to the non-linear logistic regression model proposed by Streibig (1988):

y = a/[1+(x/b)^c]

Where: *y* is the response variable (percentage control or dry mass of shoot); *x* is the dose of the herbicide (g ha^{-1}) and *a*, *b* and *c* are the estimated parameters of the equation, in which: *a* is the amplitude between the maximum point and the minimum point of the variable; *b* is the dose that provides 50% response and *c* is the slope of the curve around *b*.

The nonlinear logistic model provides an estimate of parameter C_{50} or GR_{50}. In this way, we decided to use the mathematical calculation through the inverse equation of Streibig (1988), allowing to calculate the C50, as proposed by Souza, Ferreira, Silva, Cardoso, and Ruiz (2000). The models used to obtain C50 were the same as those used in other important recent studies in relevant literature in the area (Takano et al., 2016; Takano et al., 2017; Albrecht et al. 2020b).

x=b(|a/y-1|)^(1/c)

Based on the values of C_{50} and GR_{50}, we calculated the RF = C_{50} or GR_{50} of the resistant biotype/C_{50} or GR_{50} of the susceptible biotype, noting that C_{80} and GR_{80} were also calculated. The resistance factor expresses the number of times, in which the dose required to control 50% of the resistant biotype is greater than the dose controlling 50% of the susceptible biotype (Burgos et al., 2013).

The experimental procedures, development of the dose-response curve and the statistical analysis adopted were in line with the current literature and recent publications (Zobiole et al., 2019; Albrecht et al., 2020b; Albrecht et al., 2020c). The resistance report was carried out and accepted (Heap, 2022), with wide local and international dissemination.

Conclusion

It can be concluded that *D. insularis*, is resistance to ACCase- (group A) and EPSP- (group G) inhibiting herbicides in Paraguay. Resistance to ACCase inhibitors involves the chemical groups FOP and DIM. This is the first case in the world with this particularity for the species in question, representing an alert to the scientific community and the productive system, especially about the need for constant studies on weed resistance to herbicides, as well as the urgent application of integrated weed management.

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Conflict of interests

The authors appoint that there is not any conflict of interests.

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