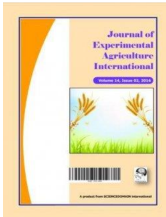

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Germination and Emergence of *Digitaria insularis* (L.) Fedde Susceptible and Resistant to Glyphosate

Silvio Douglas Ferreira[1], Jaqueline de Araújo Barbosa[1*] and Noélle Khristinne Cordeiro[1]

[1]*State University of Western Paraná, Marechal Cândido Rondon, Paraná, Brazil.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors SDF, JAB and NKC realized that all the work, since implantation, evaluations and statistical analysis, writing this paper. All authors read and approved the final manuscript.

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ABSTRACT

To Know the behavior shown in seed germination and emergence of *Digitaria insularis* (L.) Fedde enables develop strategies to reduce the seed bank in cultivated areas. The aim of this research was to evaluate the germination and emergence of weed biotypes of *D. insularis* susceptible and resistant to glyphosate in two substrates.
The experiment was divided into two stages (germination test in BOD and emergence in sand box). In both phases of the study, it was used a completely randomized experimental design, with two treatments (weed biotypes of *D. insularis* susceptible and resistant to glyphosate) and ten replications.
The work was done in the State University of Western Paraná, Brazil, Post-Graduation in Agronomy, between July 2018 and December 2018.
In the two tests the experimental units consisted of 25 seeds of *D. insularis*, evenly distributed. In the germination test was evaluated the first count and the index of germination speed. In the same way, for the emergency test was evaluated the first count and the index of emergence speed. It was also calculated the germination and the emergence percentage, total number of germinated seeds,

**Corresponding author: E-mail: jaquelineabarbosa@hotmail.com;*

total number of emerged plants.
Differences were observed in the first count, index of germination speed and germination percentage, seeds of *D. insularis* susceptible and resistant to glyphosate. During the first count, susceptible biotype showed higher germination, with 78% of compared to resistant. Similar behavior was observed for the index of germination speed and for the germination percentage, in which the susceptible biotype surpassed in 80.4% and 47%, respectively, the resistant biotype.
Conclusion: The selection of *D. insularis* biotypes resistant to glyphosate affects the germination and emergence, the species in relation to the original population of susceptible biotypes. For each 100 seeds of the biotype resistant to glyphosate 53 seeds of them germinate and 32.6 seeds can emerge.

Keywords: Weed biotypes; sour grass; weeds; bio-ecology.

1. INTRODUCTION

In Brazil has a largest number of native species of the genus *Digitaria* sp., which are weeds causing major problems in several cultures of economic expression [1]. The *species Digitaria insularis*(L.) Fedde has been the most problematic and is widely disseminated in the areas of cultivation in Brazil. The species of *D. insularis* is characterized by adaptability, aggressiveness, persistence, difficult to control, efficiency in competition for water, light and nutrients, host of pests and diseases, besides hampering the agricultural operations, such as the harvest [2,3].

The plant of *D. insularis* presents herbaceous characteristic, C4 photosynthetic metabolism, with clump, erect, fasciculate aspect and with rhizomes, height of up to 150 cm, the leaves have blades acuminate and linear with long sheath that contains a little fur and membranate ligule, presents in terminal inflorescence position with long stems and panicles branching that can measure 30 cm, the spikelets has by silky and can be oval to lanceolate [4,5]. Observed that the system of direct seeding plants of *D. insularis* showed up to six emergency flows at rates close to 30% [6].

It's known that in no-tillage system occurs the adoption of chemical management of weeds in mainly in post-emergence. However, the intensive use of desiccants in the management of the crops, has resulted in the selection of weed biotypes resistant to herbicides, such as glyphosate, if already proven in *Conyza* sp. [7], *Lolium multiflorum* [8] and *D. insularis* [4,9].

In the Paraná, the resistance of *D. insularis* biotypes resistant to glyphosate (EPSPS inhibitor) was reported in the agricultural area of several municipalities, including biotopes of agricultural areas of Cascavel and Santa Mariana, who were also not controlled with use of clethodim (ACCase inhibitor) also not controlled [10]. Before the report of resistance in *D. insularis* [11] conducted studies that amendment in the biological cycle of the resistant plants, with emphasis to the lengthening of the cycle around 21 days and the reduction in seed production of approximately 12% but wasn't studied the germination of these plants, as Pereira et al. (2017) [12] verified a higher competitive efficiency and a lower amount of tillers and inflorescences in the resistant biotype, however, in the both studies no information was reported on the germination of the seeds of these plants.

To understand the behavior the aspects related to the biology of the species as the germination and emergence of plants it's necessary to determine the best time for the application of herbicides in pre or post-emergence [13], because it's known that the germination of seeds can be defined by the influence of the physiological status and environmental conditions, being that each plant species requires a set of specific requirements for the occurrence of the germination process [14].

The knowledge of the behavior of germination and emergence of seeds of weed species (not available or summarized for several species) becomes essential for the explanation of its environmental performance in the field. Thus, the study of the germination and emergence of the seed of the biotypes of *D. insularis*, provides researchers, technicians and producers, knowledge, which can be a reference in other studies, in addition to serving as a basis for decision making in managements in the field.

In this way, the hypothesis of this study it's based on the fact that, if the selection of *D. insularis* resistant to glyphosate is modifying the growth and development, you can also change the behavior of germination and emergence of the species in relation to population of susceptible biotypes. In this context, the objective of this work was to evaluate the germination and emergence of weed biotypes of *D. insularis* susceptible and resistant to glyphosate.

2. MATERIALS AND METHODS

2.1 Experimental Details

Seeds of *D. insularis* were collected in an area of intensive use of glyphosate, due to the cultivation of soya and maize, with a history at least six application per year, as well as in open area of the use of herbicides, belonging to producers of cassava in the northern region of Paraná. The biotypes used passed by a confirmatory test of resistance, and the result was that the biotype collected in the area of use uncommon of glyphosate showed resistance factor (RF_{50}) = 7.7, compared to the other considered susceptible biotype.

The experiment was divided into two stages. The first, corresponded to the test of germination of the seeds of *D. insularis*, and was carried out in a germination chamber of the type BOD (Biochemical Oxygen Demand). The second stage corresponded to the emergency test, which was conducted in sand box.

In both stages of the study, it was used a completely randomized experimental design, with two treatments (biotypes of *D. insularis* susceptible and resistant to glyphosate) in ten replications. Both the germination test as an emergency test the experimental units consisted of 25 seeds of *D. insularis*, evenly distributed.

2.2 Methodology

The “gerbox” boxes containing two sheets of germitest paper, previously moistened with distilled water in a quantity equivalent to 2.5 times the weight of the paper. The assessments were based on the protocol established by the rule of Seed Analysis [14]. The “gerbox” boxes were placed in the chamber type BOD, with 16/8 hours photoperiod (day/night), and alternation of temperature between 30 and 20°C (day/night), as Mondo et al. [14].

For the test of emergence in sand boxes, we used plastic trays, arranged in benches with photoperiod and ambient temperature according to the methodology described by Rules for Seed Testing and Nakagawa et al. [15,16]. In the germination test was evaluated, the first count and the index of germination speed. In the same way, for the emergency test was evaluated, the first count and the index of emergence speed, using the formula proposed by Maguire [17]. It was also calculated the germination percentage, the emergence percentage, total number of germinated seeds and total number of emerged plants.

The data obtained were submitted to the *F*-test. In the occurrence of significant effects, the averages were compared by the Tukey test (P = .05) significance level.

3. RESULTS AND DISCUSSION

By analyzing the obtained results (Table 1), we observed difference in the first count, the index of germination speed and germination percentage of seeds of *Digitaria insularis* susceptible and resistant to glyphosate.

During the first count, the susceptible biotype showed higher germination, with 78% of compared to resistant. Similar behavior was observed for the index of germination speed and for the germination percentage, in which the susceptible biotype surpassed in 80.4 and 47%, respectively, the resistant biotype.

The results observed in the germination test of this work are different from those found by Nakagawa et al. [16] in studies with *D. insularis,* who found germination percentage of 96% with similar temperature conditions, however, wasn’t informed whether the biotype was used resistant or susceptible.

Already Martins [18] found that the biotype resistant to glyphosate has gained higher germination percentage in relation to susceptible, with an average of 51.3% for the resistant biotype, and 40.5% for the susceptible biotype. The authors also found the same behavior for the index of germination speed, with the resistant biotype surpassing the susceptible in 39%. However, Mendonça et al. [19], studying the germination of weed biotypes of *D. insularis* from four locations (also without defining the resistance) observed values of germination percentage that varied from 16 to 33%.

The differences in germination observed in different studies, may have relation with climatic changes suffered by plants, since, in the researches mentioned the seeds used had distinct origins. When considering the difference between the two biotypes used in this study must be taken into account that the resistant biotypes of *D. insularis* have biologic changes that extended the cycle [11], and probably may have altered the process of germination of this biotype, which justifies a lower germination percentage or abortion. In contrast, the fast germination of the susceptible biotype may be a strategy to establish itself in the environment, since this doesn't biotype resistant to chemical management.

In the evaluation of the emergency test the biotype of *D. insularis* susceptible to glyphosate was greater in all analyzed variables (Table 2).

In the first count of emerged plants was highlighted the dominance of the susceptible biotype, the difference was 109%, compared to the resistant. The disparity is even greater for the index of emergence speed, where it was observed a difference of 172.7% between susceptible and resistant. The percentage of emergence of susceptible biotype showed results very close to those observed in the germination. In this way, the susceptible biotype emerged 45.1% more than the resistant biotype.

With respect to the lowest percentage of emergence and germination increased for both species, can this directly connect to the need of light, since they are positive photoblastic [19]. In addition, Reinert [20], while studying the viability of seeds of *D. insularis*, found that these also lose viability when covered by soil.

It was evident that for the two biotypes, there was a decrease in viability, so much so that the biotypes resistant and susceptible reduced 46 and 26.74%, respectively. The biotype resisted, when submitted to sand substrate, showed greater loss of viability compared to the seeds under the paper substrate. The results observed for the total number of germinated seeds and total number of emerged plants of both biotypes corroborate with germination percentage and emergence percentage (Fig. 1A and 1B). However, the germination or emergence occurred on the sixth day, for the biotype resistant and susceptible, already resistant biotype showed germination on the seventh day and emergency only on the ninth day after sowing. This observation on the number of germination and emergence between the biotypes of *D. insularis*, shows the need for control primarily of the biotypes resistant, since these biotypes when not controlled provided increased plant population is difficult to control.

Other researchers suggest that the differences between susceptible and resistant biotypes, not only occur on germination, but continues throughout the growth and development of the plant. These differences were verified by Melo et al. [21] where the biotype resistant to glyphosate showed better performance than the susceptible biotype, with faster development and vigorous. Whereas Licorini et al. [10] found that the susceptible biotype was more precocious of the tiller to senescence.

Thus, it's evident that the use of herbicides indiscriminately and without technical criteria is selected weed biotypes resistant and causing changes that modify the behavior of these plants in relation to the original population.

It should be emphasized that the differences in germination and emergence of the biotypes of *D. insularis* susceptible and resistant to glyphosate may be used in the understanding of biological factors, ecological, genetic and management in glyphosate resistant weeds in agricultural areas, as well as in the elaboration of strategies to reduce these risks.

In this way, it becomes evident the importance of the use of strategies of integrated management of weeds in agricultural areas to avoid not only the growth of weeds, but also the selection of weed biotypes resistant to herbicides.

Table 1. Mean values of first count, the index of germination speed and germination percentage, seeds of *Digitaria insularis* susceptible and resistant to glyphosate

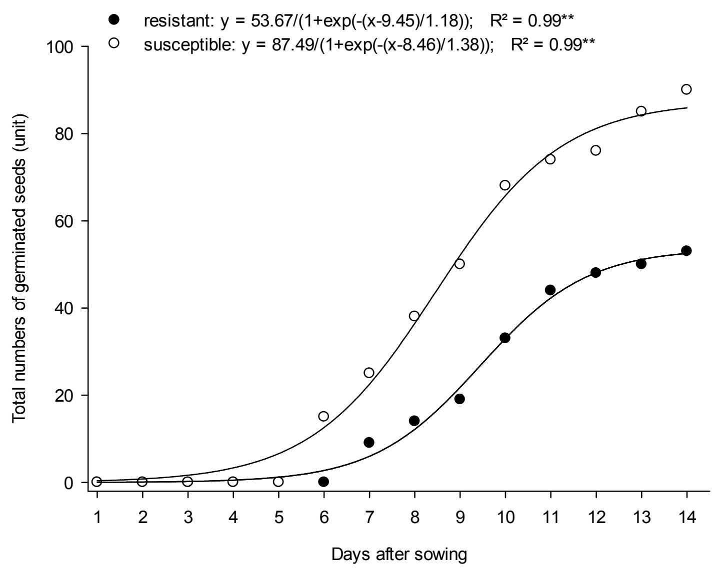
Biotypes	First count	Index of germination speed	Germination percentage
Resistant	1.00 b	3.01 b	26.00 b
Susceptible	1.78 a	5.43 a	38.22 a
CV (%):	22.45	36.76	34.99
DMS:	0.31	1.54	11.23

Medium followed by the same letter in column do not differ significantly by Tukey test (P = .01)

Table 2. Mean values of first count, index of emergence speed, emergence percentage, seeds of *Digitaria insularis* susceptible and resistant to glyphosate

Biotypes	First count	Index of emergence speed	Emergence percentage
Resistant	1.33 b	1.43 b	13.78 b
Susceptible	2.78 a	3.93 a	28.00 a
CV (%):	33.43	57.17	25.60
DMS:	0.69	1.53	5.34

Medium followed by the same letter in column do not differ significantly by Tukey test (P = .01)



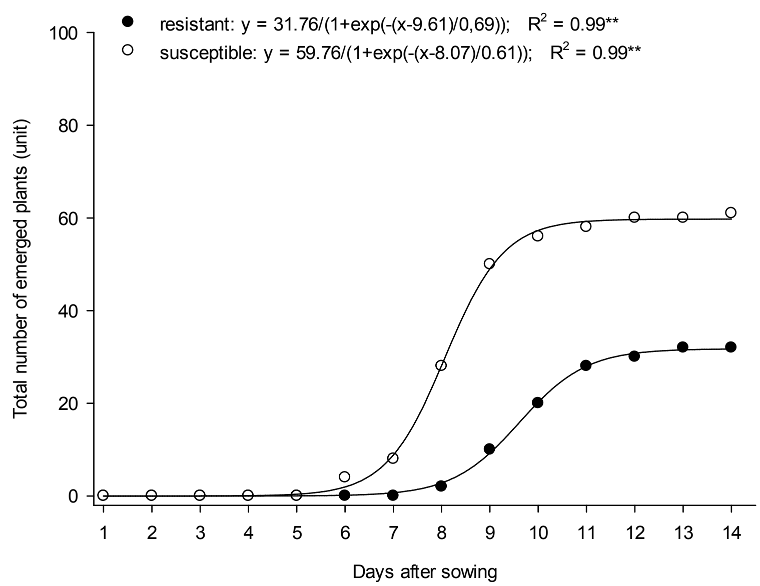


Fig. 1. Total number of germinated seeds (A) and total number of emerged plants (B) at D. insularis biotypes, resistant and susceptible to glyphosate

4. CONCLUSION

The selection of *D. insularis* biotypes resistant to glyphosate affects the germination and emergence of the species in relation to the original population of susceptible biotypes. For each 100 seeds of the biotype resistant to glyphosate 53 of them germinate and 32 can emerge, and of the biotype susceptible to glyphosate 90 of them germinate and 61 can emerge.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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