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Sixteen Years of Recurrent Selection of Ruzi Grass for Resistance to Spittlebugs (Hemiptera: Cercopidae)

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Abstract: The forage *Urochloa ruziziensis* ((R. Germ. and C.M. Evrard) Crins (synonym *Brachiaria ruziziensis*) (Poales: Poaceae)) has great potential to be adopted as pasture. However, this forage is susceptible to spittlebugs, the main insect pest of pastures in Brazil. Thus, the objective of this study was to select genotypes of *U. ruziziensis* resistant to *Deois schach* (Fabricius) and *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) through 16 cycles of recurrent selection. The resistance of 13,114 *U. ruziziensis* plants to spittlebugs was evaluated in experiments conducted between 2008 and 2023. Each plant was infested with six eggs of the insect pest at stage S4 and kept in a greenhouse for up to 40 days. After this period, surviving nymphs from the second to fifth instars were counted. The original population of *U. ruziziensis* (POP01-2008) presented a nymphal survival rate of 63%, while for the improved population (POP36-2023) the average nymphal survival rate was 32.8%. The estimated total genetic gain was 15%, and the annual genetic gain was 1%. After 16 selection cycles, in POP36-2023, approximately 63% of the genotypes (716 plants), had nymphal survival rates equal to or less than 33% and were considered resistant to *D. schach* and *M. spectabilis* nymphs.

Keywords: forage; insect pest; signal grass



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1. Introduction

Tropical forages play a fundamental role in Brazilian livestock farming, as they are used for direct grazing on approximately 100 million hectares, which corresponds to approximately 90% of the cultivated pastures in Brazil [1]. Therefore, greater efficiency in exploiting the productive potential of tropical pastures can increase the intensification of animal production and its productive capacity [2]. Forage grasses of the genus *Urochloa* play an important role in this endeavor. These plants are adapted to different types of soil and have several favorable agronomic characteristics, which make them attractive for expansion throughout the country [3–6].

The forage grass *Urochloa ruziziensis* ((R. Germ. and C.M. Evrard) Crins (synonym *Brachiaria ruziziensis*) Poales: Poaceae) is of African origin and was introduced into Brazil in 1965 by the Northern Agricultural Research and Experimentation Institute (IPEAN) [7]. This diploid species can be reproduced sexually or through clones and has high potential as a pasture due to its high nutritional quality and productivity [8]. However, this forage grass is susceptible to spittlebugs, among which the main species are *Deois schach* (Fabricius) and *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) [6,9–13].

The spittlebug nymphs feed on xylem in the plant base and produce a foamy substance utilized for self-defense. The adults live and feed on the aerial portion of the plant [14], thus reducing the chlorophyll content [15] and the plant's regrowth capacity [16]. In this context, Thompson [17] made a crude estimate that the world losses to grass feeding spittlebugs is about USD 840–2100 million year⁻¹, excluding damage in Africa and Asia, for which no estimates are possible.

To reduce this negative impact, a combination of control strategies must be carried out for the efficient management of spittlebugs; several studies have been conducted to expand these tactics [18]. According to Buitrago et al. [19], the development of new cultivars through breeding programs that incorporate host plant resistance is a long-term and low-cost strategy, with the main objective of increasing insect pest mortality in the early stages of life.

Therefore, the adoption of *U. ruziziensis* plants resistant to spittlebugs is a promising technique and the resistance characteristic is of greatest interest in tropical forage improvement programs [20]. According to Cardona and Sotelo [21], antibiosis is a type or mechanism of resistance that describes the negative effects of a resistant genotype on the biology of an insect.

To obtain cultivars resistant to spittlebugs, plant screening must be adopted through recurrent selection. This method consists of identifying resistant plants that are crossed or self-fertilized and testing and selecting their progenies; this process is repeated until populations with adequate levels of resistance are obtained [22,23]. Often, the selection and crossing process needs to be repeated for generations, as the gains in each generation can be small due to complex traits and low heritability.

In this context, considering the advantages of adopting *U. ruziziensis* in cattle feed and due to the genetic variability within populations of this forage, it is necessary to adopt recurrent selection to obtain genotypes resistant to spittlebugs. Therefore, the objective of this study was to select genotypes of *U. ruziziensis* resistant to the spittlebug species *D. schach* and *M. spectabilis* through 16 cycles of recurrent selection.

2. Materials and Methods

2.1. Location

The experiments were conducted at Embrapa Gado de Leite, in the municipality of Juiz de Fora, Minas Gerais, Brazil.

2.2. Genetic Material

A total of 13,114 *U. ruziziensis* plants were evaluated for resistance to spittlebugs in experiments conducted between 2008 and 2023 (Table 1).

A population of half-sib progenies composed of 270 plants of *U. ruziziensis* belonging to the Embrapa Gado de Leite forage improvement program provided the genotypes for the beginning of the tests, with successive annual cycles of intercrossing and selection of plants for resistance to spittlebugs throughout the period. In each selection cycle carried out within the breeding program, an average selection intensity was adopted in the range of 20% of the individuals evaluated.

Periodically, according to the needs of the improvement program, genotypes belonging to other lines of research in the program, which had been improved and evaluated for other characteristics of interest, such as productivity and forage quality, were used to introduce new genes into the genotypes that were being improved in terms of resistance to spittlebugs.

In all experiments that were carried out, the standard for susceptibility to spittlebugs was *U. decumbens* Stapf (synonym *Brachiaria decumbens*) cv. Basilisk, and the standard for resistance to spittlebugs was *U. brizantha* (Hochst. ex A. Rich.) Stapf (synonym *Brachiaria brizantha*) cv. Marandu.

Table 1. Populations of *Urochoa ruziziensis* evaluated for resistance to *Deois schach* and *Mahanarva spectabilis* from 2008 to 2023, with the experimental design and total number of plants and plants per year.

Experiment	Species of Spittlebug	Experimental Design	Number of Plants	Number of Plants/Year
POP01-2008	<i>M. spectabilis</i>	RCBD	270	270
POP02-2009	<i>M. spectabilis</i>	RCBD	148	784
POP03-2009	<i>D. schach</i>	RCBD	84	
POP04-2009	<i>M. spectabilis</i>	FABD	552	
POP05-2010	<i>D. schach</i>	RCBD	252	1645
POP06-2010	<i>M. spectabilis</i>	RCBD	430	
POP07-2010	<i>D. schach</i>	RCBD	216	
POP08-2010	<i>M. spectabilis</i>	FABD	297	
POP09-2010	<i>M. spectabilis</i>	RCBD	450	
POP10-2011	<i>M. spectabilis</i>	RCBD	180	180
POP11-2012	<i>M. spectabilis</i>	RCBD	102	636
POP12-2012	<i>M. spectabilis</i>	RCBD	216	
POP13-2012	<i>D. schach</i>	RCBD	78	
POP14-2012	<i>M. spectabilis</i>	FABD	240	
POP15-2013	<i>M. spectabilis</i>	FABD	640	1536
POP16-2013	<i>M. spectabilis</i>	RCBD	128	
POP17-2013	<i>M. spectabilis</i>	RCBD	240	
POP18-2013	<i>M. spectabilis</i>	RCBD	120	
POP19-2013	<i>M. spectabilis</i>	RCBD	408	
POP20-2014	<i>M. spectabilis</i>	RCBD	414	810
POP21-2014	<i>M. spectabilis</i>	RCBD	240	
POP22-2014	<i>M. spectabilis</i>	FABD	24	
POP23-2014	<i>D. schach</i>	FABD	132	
POP24-2015	<i>D. schach</i>	RCBD	315	1111
POP25-2015	<i>D. schach</i>	RCBD	428	
POP26-2015	<i>D. schach</i>	RCBD	368	
POP27-2016	<i>M. spectabilis</i>	FABD	200	950
POP28-2016	<i>M. spectabilis</i>	RCBD	750	
POP29-2017	<i>M. spectabilis</i>	RCBD	315	315
POP30-2018	<i>M. spectabilis</i>	RCBD	300	300
POP31-2019	<i>M. spectabilis</i>	FABD	539	629
POP32-2019	<i>M. spectabilis</i>	RCBD	90	
POP33-2020	<i>D. schach</i>	FABD	1673	1673
POP34-2021	<i>D. schach</i>	FABD	781	781
POP35-2022	<i>D. schach</i>	FABD	357	357
POP36-2023	<i>M. spectabilis</i>	FABD	1137	1137
Total plants evaluated			13,114	

Federer augmented blocks design = FABD; Randomized complete block design = RCBD.

2.3. Obtaining the Plants

The seedlings of the tested genetic materials were produced in plastic trays containing commercial substrate (Carolina Soil®—Composition: Sphagnum turf, expanded vermiculite, dolomitic calcareum, agricultural gypsum and NPK fertilizer), where the seeds were deposited and remained for approximately 40 days. The plants were subsequently transplanted into rearing units that contained a substrate composed of soil, sand, and manure in a 3:1:1 ratio.

2.4. Obtaining Pest Insect Eggs

Spittlebugs of the species *D. schach* and *M. spectabilis* collected at the Experimental Field of Embrapa Gado de Leite served as the supply of live material for the tests, which were carried out in a greenhouse.

The adults of the insect pest were collected and transferred to the Entomology Laboratory at Embrapa Gado de Leite, separated by species, and placed in cages (30 × 30 × 55 cm). The forages (*Urochloa decumbens* to *D. schach* and *Cenchrus purpureus* to *M. spectabilis*) were kept inside the cage and served as the insect's feeding substrate. Furthermore, the base of the plant and the floor of the cage were covered with hydrophilic gauze, which was used as an oviposition substrate for the spittlebugs.

Every 4 or 5 days the plants were replaced and the gauze containing the eggs was removed and subjected to a jet of water over a set of sieves. The extracted eggs were withheld through a 400-mesh sieve. Then, the eggs were individualized, counted, and transferred to Petri dishes covered with filter paper and periodically moistened with a 1% copper-sulfate-based solution. The plates containing the eggs were identified with the date and the species of spittlebug, grouped in trays, and placed in a climate-controlled chamber at 25 ± 2 °C with a 12 h photophase and a relative humidity of $70 \pm 10\%$ until the eggs reached the S4 stage, when they were close to the nymph hatching point and were taken for experiments.

2.5. Conducting the Experiments

To evaluate the nymphal survival of spittlebugs, for experiments carried out between 2008 and 2013, *U. ruziziensis* plants (the seedlings obtained as described in Section 2.3) were grown for 60 days in spittlebug rearing units made of PVC measuring 5 cm in diameter × 8 cm in height. In experiments conducted between 2014 and 2019, plants were grown in plastic pots measuring 7.5 cm in diameter × 11 cm in height. The plants evaluated between 2008 and 2019 had the surface layer of soil removed with the aid of a water jet, ensuring the exposure of the superficial roots of the plants, which were the feeding sites for the nymphs of these cercopids.

In trials conducted between 2020 and 2023, the seedlings obtained as described in Section 2.3 were maintained in the same tubes (4 cm in diameter and 14 cm in height) for 60 days, and to stimulate the growth of the roots, which were the feeding sites for the nymphs of the insect pest, the lower part of the tube containing the plant to be evaluated was inserted over an empty tube with the same dimensions.

Each plant was infested with six eggs of the insect pest at stage S4, and to prevent the nymphs from escaping, the pots were properly closed, packed in trays, and kept in a greenhouse for a period that varied between 30 and 40 days, depending on the abiotic factors of the experimental period. After this period, surviving nymphs from the second to fifth instars were counted. The plants tested against insects were never re-evaluated in subsequent experiments. Only their descendants, obtained by crossing the genotypes, were selected. The abiotic factors were recorded with the aid of a data logger and transferred to a computer using HOBOWare® version 3.7.17 software (Onset Co., Ltd., Pocasset, MA, USA). In the experimental period, the mean temperature was 25.6 °C (minimum = 15.4 °C, maximum = 45.9 °C) and the mean RH was 80.2% (minimum = 29.6%, maximum = 98.5%) (Supplementary Table S1).

2.6. Experimental Design

The experimental design was completely randomized blocks or Federer-augmented blocks, depending on the selection cycle (Table 1). Nymphal development was allowed to proceed without interference until full maturity. The survival of nymphs from the second to fifth instars was evaluated following the adapted methodology described by Cardona et al. [24], where the number of live nymphs present in each pot was recorded to calculate the percentage nymph survival, which was then used to classify the genotypes as resistant

($\leq 33\%$ survival) or susceptible ($> 33\%$). Genotypes considered resistant were selected, intercrossed, and their progenies propagated by seeds and evaluated in subsequent cycles.

2.7. Statistical Analysis

To compare the nymphal survival of *M. spectabilis* between years, the data were tested by analysis of variance and the means were compared using the Scott Knott test at 5% probability using SISVAR 5.1 software (Universidade Federal de Lavras-Minas Gerais, Brazil).

Additionally, the data also were analyzed using Henderson's linear mixed-model approach [25]. The variance components were estimated using the residual maximum likelihood method, with significance verified using the likelihood ratio test at a 5% probability. BLUE (*best linear unbiased estimator*) estimates were obtained for the fixed effects, and BLUP (*best linear unbiased predictor*) predictions were obtained for the random effects.

The individual analyses for each experiment considering the fixed control and random genotypes of the *U. ruziziensis* were carried out using the lme4 package [26] in R version 4.2.2 software (R Core Team) [27] according to the following statistical model:

$$y = 1\mu + X_t\beta_t + Z_bu_b + Z_gu_g + e, \quad (1)$$

where y is a vector of data; μ is a constant associated with the data; β_t is a vector of fixed effects of the population of regular genotypes and of the control; and u_b is a vector of block effects, where $u_b \sim N(0, I_b\sigma_b^2)$. I_b is an identity matrix of order b , where b is the number of blocks and σ_b^2 is the variance associated with the block effect; u_g is a vector of the effects of regular genotypes, where $u_g \sim N(0, I_g\sigma_g^2)$. I_g is an identity matrix of order g , where g is the number of regular genotypes of *U. ruziziensis* and σ_g^2 is the variance associated with the effect of regular genotypes; e is the vector of errors, where $e \sim N(0, I_n\sigma_e^2)$. I_n is an identity matrix of order n , where n is the amount of useful data and σ_e^2 is the variance associated with the experimental error; $1, X_t, Z_b, Z_g$ represents the vectors of 1s of order n , which are the design matrices of the effects β_t, u_b, u_g , respectively.

The following parameters were estimated from the individual analyses: the selective accuracy on genotype average (r_{gg}) using the estimator [28]: $r_{gg} = \sqrt{1 - \frac{\overline{PEV}}{\hat{\sigma}_g^2}}$, where $\overline{PEV}$ is the average variance of the prediction error associated with the genotype BLUP; the coefficient of experimental variation (CV_e) using the estimator (Pimentel-Gomes 2000) $CV_e = \frac{\sqrt{\hat{\sigma}_e^2}}{\bar{y}}$, where $\bar{y}$ is the overall mean of each experiment; and the generalized heritability (h_L^2) of [29] on the genotype mean, given by $h_g^2 = 1 - \left[\frac{v_{BLUP}}{2 \times \hat{\sigma}_g^2} \right]$, where v_{BLUP} is the average variance of the prediction error of the difference between the BLUPs of two genotypes.

Genetic progress was then estimated using the method of the Vencovsky et al. [30], which is based on genotypes from the breeding population in two successive years. The average genetic gain (GG) per year relative to the previous year was estimated using the following equation:

$$GG = \frac{\sum_{k=1}^{n-1} (\bar{y}_j - \bar{y}_i) - (\bar{y}_{c_j} - \bar{y}_{c_i})}{n - 1} \quad (2)$$

where $\bar{y}_i$ and $\bar{y}_j$ are the averages of the regular genotypes in years i and j , respectively, where $j = i + 1$ and $\bar{y}_{c_i}$ and $\bar{y}_{c_j}$ are the averages of the genotypes of the *U. ruziziensis* in years i and j , respectively.

In addition, a graphical analysis of the percentage of genotypes associated with nymphal survival that where in the range 1 (17%), 2 (33%), 3 (50%), 4 (67%), 5 (83%), and 6 (100%) live nymphs was carried out for the experiments conducted each year. With this information, for each year, the frequency of genotypes was divided into 2 groups of plants for consideration, one composed of plants that showed nymphal survival equal

to or less than 33% and the other with nymphal survival greater than 33%, following to the classification adapted established by Cardona et al. [24] for those genotypes that were considered resistant and susceptible, respectively.

3. Results

A discrepancy was observed in the nymphal survival of spittlebug nymphs fed on *U. brizantha* and *U. decumbens* plants, which were used as the resistant and susceptible controls, respectively. According to all the experiments, the average genotypic value for nymphal survival of the insects that fed on the cultivar Marandu (*U. brizantha*) was 55.18% of that observed when the host was the cultivar Basilisk (*U. decumbens*) (Table 2). The only exception was when the experiment was conducted in 2019, when the average genotypic values for nymphal survival were similar.

Table 2. Average genotypic values for nymphal survival of spittlebugs on *Urochoa ruziziensis* genotypes and resistant and susceptible control plants and estimates of genetic gain each year (year/year) or considering the first evaluation cycle (year/first year) in the period from 2008 to 2023.

Year	Average Genotypic Value $\pm$ SE			Genetic Gain (%)	
	Genotypes of <i>U. ruziziensis</i>	Standard		Year/Year	Year/First Year
		Resistant	Susceptible		
2008	63.73 $\pm$ 2.4	35.00 $\pm$ 8.03	66.67 $\pm$ 10.54	*	*
2009	43.45 $\pm$ 3.0	34.38 $\pm$ 6.57	60.90 $\pm$ 4.60	−31.81	−31.81
2010	50.80 $\pm$ 4.0	22.04 $\pm$ 5.68	50.10 $\pm$ 4.99	16.91	11.53
2011	39.05 $\pm$ 6.5	26.67 $\pm$ 9.87	58.62 $\pm$ 11.48	−23.14	−18.45
2012	50.75 $\pm$ 6.4	45.21 $\pm$ 6.34	56.71 $\pm$ 5.44	29.96	18.36
2013	58.91 $\pm$ 4.1	30.93 $\pm$ 4.49	60.43 $\pm$ 4.74	16.09	12.81
2014	51.42 $\pm$ 6.5	25.83 $\pm$ 2.69	66.76 $\pm$ 3.41	−12.72	−11.76
2015	60.84 $\pm$ 2.5	55.79 $\pm$ 7.48	76.60 $\pm$ 5.83	18.32	14.78
2016	40.55 $\pm$ 3.9	40.00 $\pm$ 7.18	75.00 $\pm$ 9.69	−33.36	−31.84
2017	29.67 $\pm$ 5.2	36.67 $\pm$ 13.33	63.33 $\pm$ 6.24	−26.81	−17.06
2018	37.00 $\pm$ 7.6	0.00 $\pm$ 0.00	26.67 $\pm$ 19.44	24.68	11.49
2019	51.55 $\pm$ 8.4	46.69 $\pm$ 5.08	46.28 $\pm$ 5.01	39.32	22.83
2020	18.02 $\pm$ 1.2	19.57 $\pm$ 4.10	35.51 $\pm$ 4.64	−65.05	−52.62
2021	23.67 $\pm$ 1.4	9.77 $\pm$ 2.93	25.29 $\pm$ 4.58	31.41	8.88
2022	42.40 $\pm$ 2.5	37.88 $\pm$ 8.44	57.58 $\pm$ 11.32	79.11	29.39
2023	32.83 $\pm$ 2.2	14.35 $\pm$ 5.79	44.91 $\pm$ 7.04	−22.59	−15.03
Average	43.41 $\pm$ 4.24	30.05 $\pm$ 6.13	54.46 $\pm$ 7.44		
Genetic gain				−1.00	−15.00

* Initial plant population—no data to estimate gain. For genetic gain, negative values (−) indicate a reduction in the survival of the insect pest and positive values (+) indicate an increase in the survival of the insect pest in the evaluated population.

The general averages, considering all the experiments carried out during the study period, for the genotypic value for nymphal survival of *D. schach* and *M. spectabilis* nymphs were 43.41% when fed on the different genotypes of *U. ruziziensis* and 30.05% and 54.46% for the resistant and susceptible controls, respectively. Even though it is considered resistant, the Marandu cultivar provided conditions for the development of insects (Table 2), but the average survival rate was less than 33%.

In each of the experiments conducted in different years, variability was observed between the genotypes of *U. ruziziensis* that arose from different selection cycles ($F = 124.6$; $p < 0.0001$, $df = 15$). In the first trial, carried out in 2008, a 41 to 100% variation in the nymphal survival of the insect pest was observed, with an average of 63.72% for the genotypic value for nymphal survival, which was the highest average for the period from 2008 to 2023, characterized as a population of plants susceptible to spittlebugs (Table 2, Figure 1).

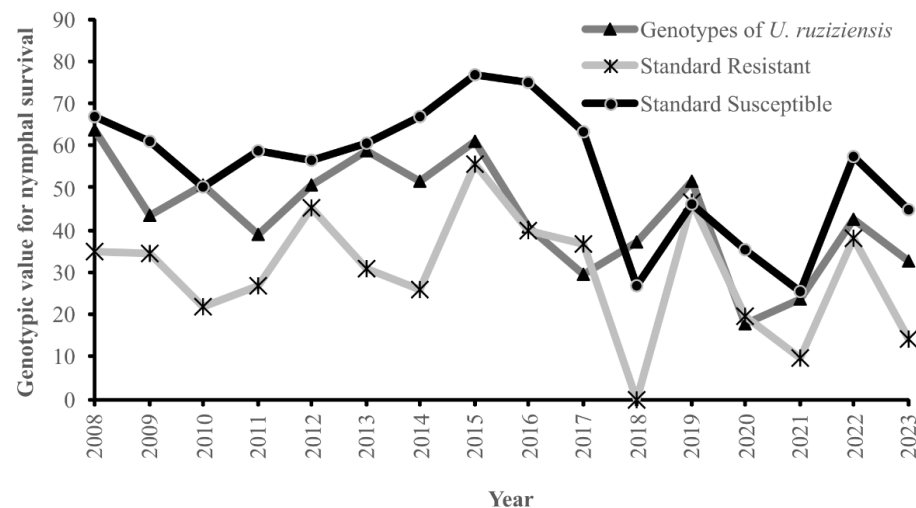


Figure 1. Average genotypic value for the nymphal survival of spittlebugs in genotypes of *Urochloa ruziziensis*, the resistance standard, and the susceptible standard evaluated over 16 cycles of recurrent selection.

In successive experiments conducted over the years, a reduction in nymphal survival was observed, demonstrating the success of the selection of *U. ruziziensis* plants, given that the improved plants were unfavorable to the development of insects in each cycle. The lowest averages ($F = 124.6$; $p < 0.0001$; $df = 15$) for nymphal survival were observed in assessments carried out in 2020 and 2021, followed by 2017, 2018, and 2023 (Table 1, Figure 1). Although the average genotypic value for the nymphal survival of the improved genotypes was always lower than that of the initial plant population, the sets of plants evaluated in 2010, 2012, 2013, 2015, 2018, 2019, 2021, and 2022 showed increases in the average genotypic value of nymphal survival in relation to the population tested in the previous year (Table 2, Figure 1).

According to the data from the first and last years, there was a reduction of 51.49% in the genotypic value of survival spittlebug nymphs that fed on *U. ruziziensis* plants. In other words, the original population presented a genotypic value of 63.73%, while for the improved population this value was 32.83% on average (Table 2, Figure 1).

Given the genetic variability of *U. ruziziensis*, it was possible to estimate the genetic gain from selection using the genotypic value for the nymphal survival of spittlebug nymphs (Table 2). The heritability (h^2g) was estimated and varied from 38.40 to 12.20 for the genotypic values of nymphal survival in the *U. ruziziensis* genotypes tested between 2008 and 2023. The selective accuracy values were moderate to high in 80% of the evaluated population.

Excluding the environmental component present in the evaluations, the estimate of the genetic gain with the selection of plants less favorable to the development of spittlebug nymphs was 15%, with a 1% gain per year (Table 2).

The increase in the number of individuals with the desired phenotype was also observed graphically, as shown in Figures 2 and 3. In the first evaluation, which was carried out in 2008, no plants were observed in the 16.7 and 33% nymphal survival classes. In contrast, according to the data from the 2023 population assessments, the majority of the tested plants exhibited this phenotype, that is, low insect survival. More than 50% of the plants evaluated in the first 8 years of selection, that is, between 2008 and 2015, had nymphal survival greater than 33%. In populations evaluated between 2016 and 2023, excluding only the population evaluated in 2019, more than 50% of the tested genotypes had nymphal survival rates less than 33%, reinforcing the greater possibility of identifying and selecting resistant materials in improved populations due to the increase in the frequency of resistance genes to spittlebugs (Figure 2). Recurrent selection contributed to a considerable increase in the number of genotypes resistant to the insect pest, considering

that after 16 selection cycles in POP36-2023, approximately 63% of the genotypes, a total of 716 plants, had nymphal survival equal to or less than 33% (Figures 2 and 3).

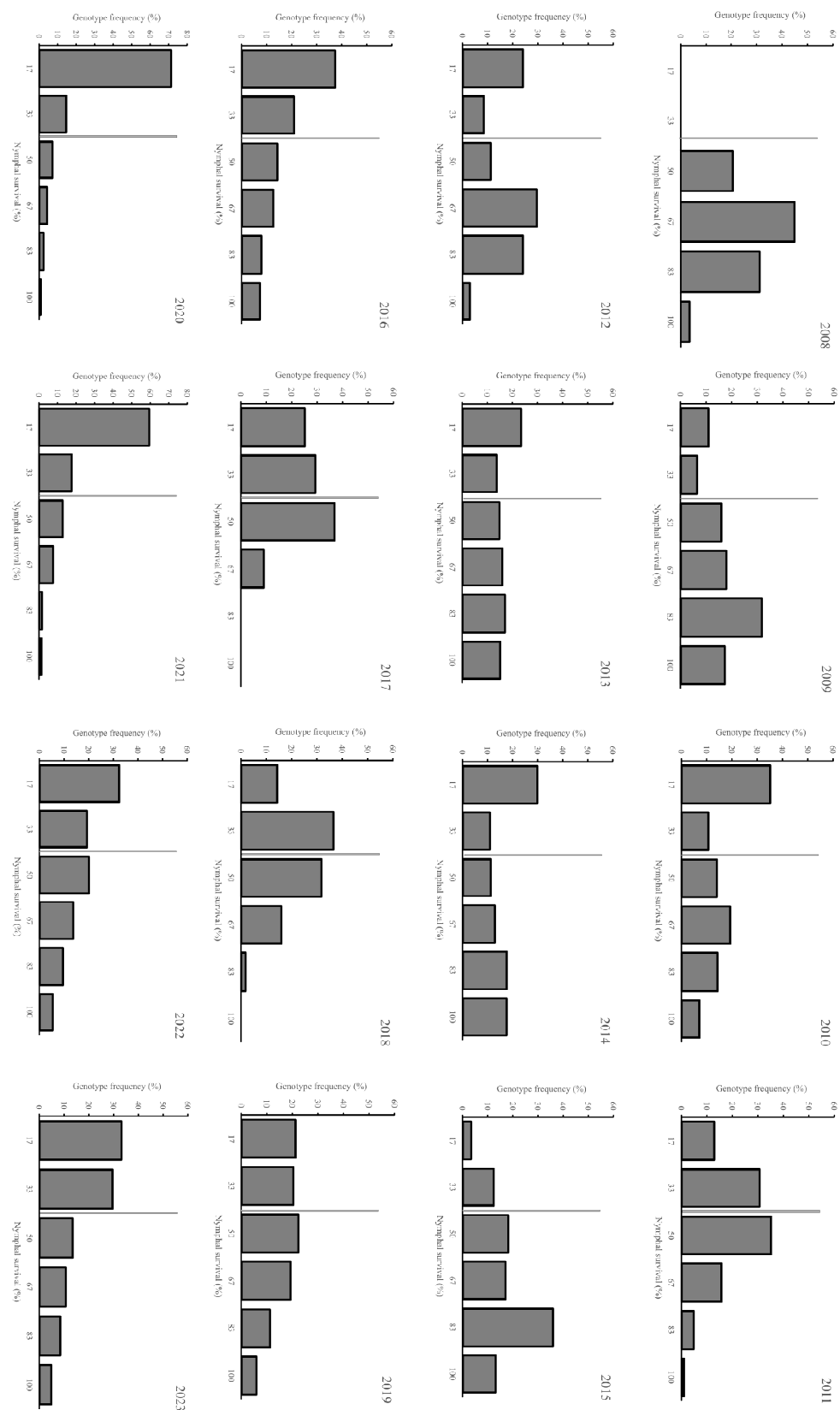


Figure 2. Annual frequency distribution of genotypes (%) of *Urochloa ruziziensis* with nymphal survival greater than 33% (susceptible) or equal to or less than 33% (resistant) between 2008 and 2023.

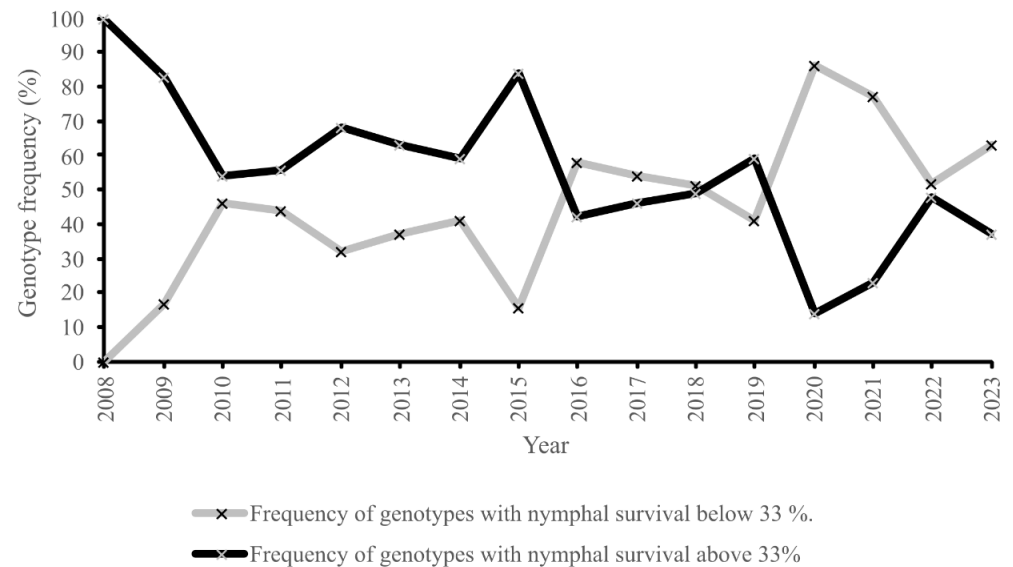


Figure 3. Frequency distribution of genotypes (%) of *Urochloa ruziziensis* with nymphal survival greater than 33% (susceptible) or equal to or less than 33% (resistant).

4. Discussion

Although *U. ruziziensis* has good nutritional quality and is accepted by livestock, this forage species was practically abandoned by rural producers at the end of the last century. Among the main reasons for this abandonment was its susceptibility to spittlebugs [31]. In the early 2000s, however, *U. ruziziensis* began to be cultivated again, its planted area annually expanding, and is currently the main forage species used in integrated cultivation systems involving crops, livestock, and forestry (ILPF) [31]. As it is fully diploid and sexual, unlike other species of the genus, the generation and use of genetic variability is more viable. This approach facilitates and favors genetic improvement, which has achieved considerable gains for different traits of forage importance, based on the phenotypic recurrent selection strategy. Even for resistance to spittlebugs, the main pest that attacks this forage in Brazil, the results of the selection cycles were promising [32].

With this plant improvement strategy, it is possible to evolve from a population of plants susceptible to spittlebugs to an improved population in which more than 60% of the plants are considered resistant to the insect pest (Figures 2 and 3). Cardona et al. [24] studied the resistance of the genus *Urochloa* to spittlebugs and established that plants with a nymphal survival percentage less than 33% should be considered resistant to this pest. This category includes, for example, the cultivar Marandu (*U. brizantha*), which is considered a standard of resistance to spittlebugs in Brazilian growing conditions. In the first assessment of resistance to spittlebugs, which was carried out in 2008, 100% of the genotypes showed nymphal survival greater than 33%. After 16 selection cycles, approximately 63% of the plants showed nymphal survival less than 33% and were considered resistant to spittlebugs (Figures 1–3).

The average genotypic values for nymphal survival obtained for the Basilisk and Marandu cultivars reinforced the idea of them being representative cultivars of susceptibility and resistance to spittlebugs, respectively, and demonstrated that these cultivars were suitable for use as standards in our study (Table 2, Figure 1). These results reinforce the efficiency of the evaluations and the ability of the methodology to detect differences in the genotypic value for the nymphal survival of spittlebug nymphs in host *Urochloa* plants over 16 cycles of recurrent selection.

Similar results showing increases in resistance to spittlebugs for *Urochloa* species via recurrent phenotypic selection have already been reported by other authors [33–40]. Employing this same improvement strategy, Miles et al. [34] managed to identify promising *Urochloa* plants in terms of resistance to spittlebugs, starting from the fifth selection cycle.

In the current work, in the second selection cycle, it was verified that almost 20% of the evaluated genotypes were considered resistant to spittlebug nymphs, and from the eighth selection cycle onward, at least 50% of the evaluated materials showed nymphal survival less than 33% and were therefore considered resistant. In this context, it is important to highlight the unprecedented fact of this research, in which 16 cycles of recurrent selection of *U. ruziziensis* were conducted, increasing the reliability of the genotypes selected as resistant to spittlebugs.

According to Miles et al. [34], there is a need for subsequent cycles to increase favorable alleles for resistance to spittlebugs. This increase in favorable alleles within the improved population makes it possible, in practice, to more easily identify individuals who present the desired phenotypic characteristics as they become more frequent. This fact is evidenced in the present work, in which in subsequent cycles of recurrent selection, the evaluated genotypes promoted a considerable reduction in the survival of *D. schach* and *M. spectabilis* nymphs. The recurrent selection strategy has been used successfully in several crops with varied objectives, always aiming to increase the allelic frequency and, consequently, favorable genotypes within the improved population [41–44].

The heritability was considered moderate-to-high according to the classification proposed by Resende 2002 [45]. Heritability indicates the degree by which individuals pass their traits over the generations and can also imply something about the genetic control of traits. In this sense, a high heritability value indicates that there is a good chance of genetic gain from selection [46].

The mean heritability for clones from the initial population was 38.40% for the average genotype value for nymphal survival, showing that the variability of plant resistance traits against *D. schach* and *M. spectabilis* can be transferred to subsequent generations. Also, these results indicate the efficiency of selection within *U. ruziziensis* for plant resistance traits. Similarly, Silva et al. [47] reported a high heritability for the resistance traits of *U. ruziziensis* against *Collaria oleosa* (Hemiptera: Miridae).

According to Juhász et al. [48], accuracy refers to the correlation between the expected genetic values and the true genetic value of an individual. The higher the accuracy to a given individual, the higher the reliability of the assessment and the expected genetic value, as well as the higher the gain from selection. In the current work, we estimated a value above 60% for the genotypic values of nymphal survival in the *U. ruziziensis* genotypes tested, reinforcing the reliability of the results.

The estimated gain from selection is influenced, among other factors, by the heritability of the trait under consideration and the intensity of selection applied to each cycle [42,49–51]. In all selection cycles carried out within the breeding program, an average selection intensity was adopted, in the range of 20% of the individuals evaluated. This association led to an estimate of genetic gain with selection of 1% per year, that is, a 15% gain in the period evaluated (Table 2), indicating that within the improved population, there is, over time, an increase in the frequency of favorable alleles related to resistance to cercopid nymphs favoring their selection in future cycles.

Recurrent phenotypic selection is a method that allows the insertion of new sources of variability throughout breeding cycles [42,50]; this method is efficient for the improvement of forage plants and can be verified in the working population of *U. ruziziensis* under evaluation in this work. The genetic improvement of any plant species aims to increase different characteristics to obtain a product that meets demand, and different characteristics generally increase in each species. These different characteristics can occur at the same time or in isolation [50,52].

When selection is carried out for different traits at the same time, normally, the gains obtained for each trait are smaller than they would be if they were carried out individually, even if heritability is high [50,52]. The improvement of *U. ruziziensis* aims to obtain cultivars that combine high production, high biomass quality, and tolerance to the main abiotic stresses, such as drought, cold, and toxic aluminum, and biotic stresses [32]. As a result, the estimated gains from selection for resistance to the insect pest in this work were not

linear over the years (Figure 1). Although unfavorable, these results were expected within the breeding strategy used, as recurrent selection was not based solely on resistance to the insect and there was interest in introducing other alleles into the population.

It was observed that in years in which greater emphasis was placed on the selection of plants resistant to spittlebugs, the gains obtained for this characteristic were more pronounced, which was the case for populations evaluated between 2008 and 2009, 2016 and 2017, and 2019 and 2020. Conversely, when selection did not prioritize this characteristic or new genotypes were inserted into the working population aiming to increase the genetic variability, the gain estimates for resistance to spittlebugs were unfavorable and there was an increase in the nymphal survival of the insect pest. Similar results for a reduction in the average number of populations improved by the introduction of genetic variability from germplasm banks or other sources have been commonly noted in different crops [52,53].

According to Souza Sobrinho et al. [31], improved forages contribute to the food security of livestock by reducing the chances of limited forage availability due to pest attacks and by providing alternative cultivars, thereby reducing the genetic vulnerability that occurs when cultivating large areas with just one or a few forage species/cultivars. It is therefore suggested that the improved population in the current research be subjected to a new stage, in which it will be cloned and subjected to attack by the insect pest in order to confirm the resistance of *U. ruziziensis*, guaranteeing the producer a reduction in the problems caused by pasture leafhoppers.

It can be observed from the results of this work that the gains from selection for resistance to pasture spittlebugs in *U. ruziziensis* are considerable and of great relevance for this forage species. Therefore, the strategy adopted by the *U. ruziziensis* breeding program is efficient, resulting in gains for most of the characteristics evaluated throughout the cycles. In the current improved population (POP36-2023), new cultivars that combine important forage characteristics, such as resistance to spittlebugs, are expected, which seemed impossible or at least unlikely a few years ago.

5. Conclusions

Thus, after 16 cycles of recurrent selection, it became possible to identify 716 genotypes of *U. ruziziensis* with nymphal survival rates less than 33%, that is, those resistant to *D. schach* and *M. spectabilis*, enabling the development of a cultivar resistant to these spittlebugs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14071516/s1>, Table S1: Abiotic factors of the greenhouse where the experiments were conducted.

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References

1. Simeão, R.; Silva, A.; Valle, C.; Resende, M.D.; Medeiros, S. Genetic Evaluation and Selection Index in Tetraploid *Brachiaria ruziziensis*. *Plant Breed.* **2016**, *135*, 246–253. [\[CrossRef\]](#)
2. Martha, G.B., Jr.; Alves, E.; Contini, E. Land-Saving Approaches and Beef Production Growth in Brazil. *Agric. Syst.* **2012**, *110*, 173–177. [\[CrossRef\]](#)
3. Wenzl, P.; Patiño, G.M.; Chaves, A.L.; Mayer, J.E.; Rao, I.M. The High Level of Aluminum Resistance in Signal-grass Is Not Associated with Known Mechanisms of External Aluminum Detoxification in Root Apices. *Plant Physiol.* **2001**, *125*, 1473–1484. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Wenzl, P.; Mancilla, L.I.; Mayer, J.E.; Albert, R.; Rao, I.M. Simulating Infertile Acid Soils with Nutrient Solutions. *Soil Sci. Soc. Am. J.* **2003**, *67*, 1457–1469. [\[CrossRef\]](#)
5. Rao, I.; Miles, J.W.; García, R.; Ricaurte, J. Selección de Híbridos de *Brachiaria* Con Resistencia a Aluminio. *Pasturas Trop.* **2006**, *28*, 20–25.
6. Souza Sobrinho, F.; Auad, A.M.; Lédo, J.F.S. Brazilian Society of Plant Breeding. Printed in Brazil Genetic Variability in *Brachiaria ruziziensis* for Resistance to Spittlebugs. *Crop Breed. Appl. Biotechnol.* **2010**, *10*, 83–88. [\[CrossRef\]](#)
7. Serrão, E.A.S.; Neto, M.S. *Informações Sobre Duas Espécies de Gramíneas Forrageiras Do Gênero Brachiaria Na Amazônia: B. Decumbens Stapf e B. ruziziensis Germain et Everard*; Instituto de Pesquisa e Experimentação Agropecuária do Norte: Belém, Brazil, 1971.
8. Pessoa-Filho, M.; Azevedo, A.L.S.; Sobrinho, F.S.; Gouvea, E.G.; Martins, A.M.; Ferreira, M.E. Genetic Diversity and Structure of Ruzigrass Germplasm Collected in Africa and Brazil. *Crop Sci.* **2015**, *55*, 2736–2745. [\[CrossRef\]](#)
9. Keller-Grein, G.; Maass, B.L.; Hanson, J. Natural Variation in *Brachiaria* and Existing Germplasm Collections. In *Brachiaria: Biology, Agronomy, and Improvement*; Miles, J.W., Maass, B.L., Valle, C.B., Eds.; Centro Internacional de Agricultura Tropical: Cali, Colombia, 1996; pp. 16–42.
10. Lascano, V.P.B.; Euclides, C.E. Nutritional Quality and Animal Production of *Brachiaria* Pastures. In *Brachiaria: Biology, Agronomy, and Improvement*; Miles, J.W., Maass, B.L., Valle, C.B., Eds.; Centro Internacional de Agricultura Tropical: Cali, Colombia, 1996; pp. 106–123.
11. Schöbel, C.; Carvalho, G.S. Niche Modeling of Economically Important *Mahanarva* (Hemiptera, Cercopidae) Species in South and Central America: Are Brazilian Spittlebug Sugarcane Pests Potential Invaders of South and Central America? *J. Econ. Entomol.* **2019**, *113*, 115–125. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Souza Sobrinho, F. Melhoramento de Forrageiras No Brasil. In *Forragicultura e Pastagens: Temas em Evidência*; Evangelista, A.R., Amaral, P.N.C., Padovani, R.F., Tavares, V.B., Salvador, F.M., Peron, A.J., Eds.; UFLA: Lavras, Brazil, 2005; pp. 65–120.
13. Sotelo; Paola, A.; Maria, F.; Miller, A.F.; Cardona, C.; Miles, J.W.; Sotelo, P.A.; Sotelo, G.; Montoya, J. Sublethal Effects of Antibiosis Resistance on the Reproductive Biology of Two Spittlebug (Hemiptera: Cercopidae) Species Affecting *Brachiaria* spp. *J. Econ. Entomol.* **2008**, *101*, 564–568. [\[CrossRef\]](#)
14. Valério, J.; Cardona, C.; Peck, D.; Sotelo, G. Spittlebugs: Bioecology, Host Plant Resistance and Advances in IPM. In Proceedings of the International Grassland Congress, Sao Paulo, Brazil, 11–21 February 2001; FEALQ: Piracicaba, Brazil, 2001; pp. 217–221.
15. Resende, T.T.; Auad, A.M.; Fonseca, M.G. How Many Adults of *Mahanarva spectabilis* (Hemiptera: Cercopidae) Should Be Used for Screening *Brachiaria ruziziensis* (Poales: Poaceae) Resistance? *J. Econ. Entomol.* **2014**, *107*, 396–402. [\[CrossRef\]](#)
16. Aguiar, D.D.M.; Auad, A.M.; Fonseca, M.G.; Leite, M.V. *Brachiaria ruziziensis* Responses to Different Fertilization Doses and to the Attack of *Mahanarva spectabilis* (Hemiptera: Cercopidae) Nymphs and Adults. *Sci. World J.* **2014**, *2014*, 543813. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Thompson, V. Associative Nitrogen Fixation, C 4 Photosynthesis, and the Evolution of Spittlebugs (Hemiptera: Cercopidae) as Major Pests of Neotropical Sugarcane and Forage Grasses. *Bull. Entomol. Res.* **2004**, *94*, 189–200. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Auad, A.M.; de Carvalho, C.A.; da Silva, D.M.; Deresz, F. Flutuação Populacional de Cigarrinhas-Das-Pastagens Em Braquiária e Capim-Elefante. *Pesqui. Agropecu. Bras.* **2009**, *44*, 1205–1208. [\[CrossRef\]](#)
19. Buitrago, P.A.E.; Manzano, M.R.; Hernández, L.M. Spittlebugs (Hemiptera: Cercopidae): Integrated Pest Management on Gramineous Crops in the Neotropical Ecozone. *Front. Sustain. Food Syst.* **2022**, *6*, 891417. [\[CrossRef\]](#)
20. Pereira, J.F.; Azevedo, A.L.S.; Pessoa-Filho, M.; Romanel, E.A.D.C.; Pereira, A.V.; Vigna, B.B.Z.; Souza, F.D.; Benites, F.R.G.; Ledo, F.J.D.S.; Brito, G.G.D.; et al. Research Priorities for Next-Generation Breeding of Tropical Forages in Brazil. *Crop Breed. Appl. Biotechnol.* **2018**, *18*, 314–319. [\[CrossRef\]](#)
21. Cardona, C.; Sotelo, G. Mecanismos de Resistencia a Insectos: Naturaleza e Importancia En La Formulacion de Estrategias de Mejoramiento Para Incorporar Resistencia a Salivazo En *Brachiaria*. *Pasturas Trop.* **2005**, *27*, 2.
22. Barnes, D.K.; Goplen, B.P.; Baylor, J.E. Highlights in the USA and Canada. In *Alfalfa and Alfalfa Improvement*; Hanson, A.A., Barnes, D.K., Jr., Hill, R.R., Eds.; American Society of Agronomy: Madison, WI, USA, 1988.
23. Casler, M.D.; Pedersen, J.F.; Eizenga, G.C.; Stratton, S.D. Germplasm and Cultivar Development. In *Cool-Season Forage Grasses*; Moser, L.E., Buxton, D.R., Casler, M.D., Eds.; American Society of Agronomy: Madison, WI, USA, 1996.
24. Cardona, C.; Miles, J.W.; Sotelo, G. An Improved Methodology for Massive Screening of *Brachiaria* spp. Genotypes for Resistance to *Aeneolamia varia* (Homoptera: Cercopidae). *J. Econ. Entomol.* **1999**, *92*, 490–496. [\[CrossRef\]](#)

25. Mrode, R.; Thompson, R. *Linear Models for the Prediction of Animal Breeding Values*; Mrode, R., Ed.; CABI: Oxfordshire, UK, 2014; ISBN 9781780643915.
26. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using Lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [\[CrossRef\]](#)
27. R Core Team. *R: A Language and Environment for Statistical Computing*; Foundation for Statistical Computing: Vienna, Austria, 2022.
28. Resende, M.D.V.; Duarte, J.B. Precisão e Controle de Qualidade Em Experimentos de Avaliação de Cultivares. *Pesqui. Agropecuária Trop.* **2007**, *37*, 182–194.
29. Cullis, B.R.; Smith, A.B.; Coombes, N.E. On the Design of Early Generation Variety Trials with Correlated Data. *J. Agric. Biol. Environ. Stat.* **2006**, *11*, 381–393. [\[CrossRef\]](#)
30. Vencovsky, R.; Moraes, A.; Garcia, J.; Teixeira, N. Progresso Genético Em Vinte Anos de Melhoramento Do Milho No Brasil. In *Proceedings of the 9º Congresso de Milho e Sorgo, Anais*; EMBRAPA-CNPMS: Sete Lagoas, Brazil, 1986; pp. 300–307.
31. Souza Sobrinho, F.; Auad, A.M.; Santos, A.M.B.; Gomide, C.A.M.; Martins, C.E.; Castro, C.R.T.; Paciullo, D.S.C.; Benites, F.R.G.; Rocha, W.S.D. *Boletim Técnico*; Embrapa: Juiz de Fora, Brazil, 2022.
32. Souza Sobrinho, F.; Benites, F.R.G. Melhoramento Genético de *Brachiaria ruziziensis*: Histórico e Estratégias. In *Tópicos Especiais em Ciência Animal IV*; Martins, C.B., Deminici, B.B., Moreira, G.R., Mendonça, P.P., Eds.; CAUFES: Alegre, Brazil, 2016; pp. 309–329.
33. Cardona, C.; Fory, P.; Sotelo, G.; Pabon, A.; Diaz, G.; Miles, J.W. Antibiosis and Tolerance to Five Species of Spittlebug (Homoptera: Cercopidae) in *Brachiaria* spp.: Implications for Breeding for Resistance. *J. Econ. Entomol.* **2004**, *97*, 635–645. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Miles, J.W.; Cardona, C.; Sotelo, G. Recurrent Selection in a Synthetic *Brachiaria* grass Population Improves Resistance to Three Spittlebug Species. *Crop Sci.* **2006**, *46*, 1088–1093. [\[CrossRef\]](#)
35. Auad, A.M.; Resende, T.T.; Santos, D.R.; Souza Sobrinho, F.; Fonseca, M.G.; Madalena, I.S.P. Seleção de Clones de *Brachiaria ruziziensis* resistentes à *Deois flavopicta* (Hemiptera: Cercopidae). In *Proceedings of the 48a Reunião Anual da Sociedade Brasileira de Zootecnia*, Belém, Brazil, 18–21 July 2011.
36. Auad, A.M.; Souza Sobrinho, F.; Resende, T.T.; Benites, F.R.G. Seleção de Clones de *Brachiaria ruziziensis* Quanto a Resistência à *Mahanarva Spectabilis*. In *Proceedings of the XXII Congresso de Pós graduação da UFLA*, Lavras, Brazil, 14–18 October 2013; p. 4.
37. Auad, A.M.; Resende, T.T.; Souza Sobrinho, F.; Toledo, A.M.O.; Lucindo, T.S. Identificação De *Brachiaria ruziziensis* Resistentes à *Mahanarva spectabilis* (Hemiptera: Cercopidae): Quarto Ciclo de Seleção. In *Proceedings of the XXXVII Semana de Biologia da UFJF*, Juiz de Fora, Brazil, 10–14 November 2014.
38. Auad, A.M.; Souza Sobrinho, F.; Fonseca, M.G.; Resende, T.T.; Parchen, H.A.; Rodrigues, B.S.; Lucindo, T.S. Seleção de Populações de *Brachiaria ruziziensis* (Germain & Edvard) Quanto à Resistência a *Deois schach* (Fabricius, 1787) (Hemiptera: Cercopidae). In *Proceedings of the Semana De Biologia Da UFJF*, Juiz de Fora, Brazil, 19–25 October 2015.
39. Auad, A.M.; Resende, T.T.; Souza Sobrinho, F.; Silva, S.E.B.; Claudino, S.S.; Rodrigues, B.S. Seleção de Genótipos de *Brachiaria ruziziensis* (Germain & Evrard) Resistentes à *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae). In *Proceedings of the XXV Congresso de Pós Graduação da UFLA*, Lavras, Brazil, 22–27 November 2016.
40. Toledo, A.M.O.; Auad, A.M.; Fonseca, C.S.; Souza Sobrinho, F.; Resende, T.T.; Rodrigues, B.S.; Borges, R.A. Seleção de Genótipos de *Brachiaria ruziziensis* (Germain & Edvard) Resistentes à *Deois schach* (Fabricius, 1787) (Hemiptera: Cercopidae). In *Proceedings of the Semana de Biologia da UFJF*, Juiz de Fora, Brazil, 19–25 October 2015; pp. 1–3.
41. Berilli, A.P.C.G.; Pereira, M.G.; dos Trindade, R.S.; da Costa, F.R.; Cunha, K.S.D. Resposta a Seleção No 11o Ciclo de Seleção Recorrente Recíproca Entre Famílias de Irmãos completos de Milho. *Acta Sci. Agron.* **2013**, *35*, 435–441. [\[CrossRef\]](#)
42. Ramalho, M.A.P.; Santos, J.B.; Pinto, C.A.B.P. *Genética Na Agropecuária*, 2nd ed.; UFLA: Lavras, Brazil, 2000.
43. Pereira de Castro, A.; Breseghello, F.; Furtini, I. Population Improvement via Recurrent Selection Drives Genetic Gain in Upland Rice Breeding. *Heredity* **2023**, *131*, 201–210. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Fritsche-Neto, R.; Sabadin, F.; DoVale, J.C.; Borges, K.L.R.; de Souza, P.H.; Crossa, J.; Garbuglio, D.D. Realized Genetic Gains via Recurrent Selection in a Tropical Maize Haploid Inducer Population and Optimizing Simultaneous Selection for the next Cycles. *Crop Sci.* **2023**, *63*, 2865–2876. [\[CrossRef\]](#)
45. Resende, M.D.V. *Software Selegen-Reml/Blup*; Embrapa Floresta: Colombo, Brazil, 2002; p. 67.
46. Missio, R.F.; Dias, L.A.S.; Moraes, M.L.T.; Resende, M.D.V. Selection of *Pinus caribaea* Var. Bahamensis Progenies Based on the Predicted Genetic Value. *Crop Breed. Appl. Biotechnol.* **2004**, *4*, 399–407. [\[CrossRef\]](#)
47. Silva, D.M.; de Moraes, J.C.; Auad, A.M.; das Fonseca, M.G.; Souza Sobrinho, F. Genetic Variability of *Brachiaria ruziziensis* Clones to *Collaria oleosa* (Hemiptera: Miridae) Based on Leaf Injuries. *Am. J. Plant Sci.* **2013**, *4*, 2418–2424. [\[CrossRef\]](#)
48. Juhász, A.C.P.; de Moraes, D.L.B.; Soares, B.O.; Pimenta, S.; de Rabello, H.O.; de Resende, M.D.V. Parâmetros Genéticos e Ganho Com a Seleção Para Populações de Pinhão Manso (*Jatropha curcas*). *Pesqui. Florest. Bras.* **2010**, *30*, 25–35. [\[CrossRef\]](#)
49. Vencovsky, R.; Barriga, P. *Genética Biométrica No Fito Melhoramento*; Sociedade Brasileira de Genética: Ribeirão Preto, Brazil, 1992.
50. Borém, A. *Melhoramento de Plantas*, 20th ed.; UFV: Viçosa, Brazil, 1997.
51. Cruz, C.D.; Carneiro, P.C. *Modelos Biométricos Aplicados Ao Melhoramento de Plantas*; Ceres: Viçosa, Brazil, 2003.

52. Fradgley, N.; Gardner, K.A.; Bentley, A.R.; Howell, P.; Mackay, I.J.; Scott, M.F.; Mott, R.; Cockram, J. Multi-Trait Ensemble Genomic Prediction and Simulations of Recurrent Selection Highlight Importance of Complex Trait Genetic Architecture for Long-Term Genetic Gains in Wheat. *Silico Plants* **2023**, *5*, diad002. [[CrossRef](#)]
53. Benites, F.R.G.; Pinto, C.A.B.P. Genetic Gains for Heat Tolerance in Potato in Three Cycles of Recurrent Selection. *Crop Breed. Appl. Biotechnol.* **2011**, *11*, 133–140. [[CrossRef](#)]

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