

Ploidy level and chemical control of two *Paspalum urvillei* Steud. Populations

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Abstract: Background: *Paspalum urvillei* Steud. (vaseygrass) is an important Poaceae species in several countries. This species is native to Brazil; however, it has become a troublesome weed, especially in pasture areas in Florida, USA. Vaseygrass is botanically similar to forage species in Brazil, including *Paspalum notatum*, *Paspalum dilatatum*, and *Urochloa brizantha*. Therefore, herbicide options to control vaseygrass are limited. Also, polyploid grasses often possess enhanced herbicide detoxification capacity, which can affect control of this species.

Objective: Determine the ploidy level and evaluate chemical control of two populations of vaseygrass historically exposed to herbicide selection pressure.

Methods: The ploidy level was evaluated using flow cytometry, and the chemical control was assessed using five herbicide treatments in

two experiments: Control, imazapic, nicosulfuron, glyphosate, and nicosulfuron + glyphosate. Visible injury, plant height, culm diameter, number of leaves, total relative chlorophyll content, number of tillers, and dry matter were evaluated.

Results: The ploidy analysis revealed that both populations had similar DNA content and were classified as tetraploids. The average DNA content in accessions of vaseygrass was 2.31 pg for *Fence* and 2.25 pg for *Pasture*. Considering visible injury, glyphosate and nicosulfuron + glyphosate caused 65% at 7 days after treatment.

Conclusions: The two populations *P. urvillei* are tetraploids. Glyphosate alone or mixed with nicosulfuron are the best herbicide options to control *P. urvillei*.

Keywords: DNA Quantification; Weed Selection Pressure; Flow Cytometry; Pasture Management; Vaseygrass; Invasive Species.

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1. Introduction

In the Poaceae family, the genus *Paspalum* L. stands out as one of the largest, with approximately 350 species. Most *Paspalum* species are native to Tropical and Subtropical America, such as *Paspalum urvillei* Steud. (vaseygrass). This plant is native to the Pampa biome in Rio Grande do Sul state, Brazil. It is not used as a forage grass but is commonly used in genetic breeding programs (Burson, 1979; Maciel et al., 2009). Vaseygrass is a perennial C4 grass which can grow up to 220cm tall with small spikelets between 1.8–2.8 mm and produces 231 to 1,267 seeds per plant (Jeffries et al., 2017; Lee et al., 2013; Lopes, Franke, 2011).

This species has become a common weed in pastures, roadside, crops, and other areas, due to its aggressiveness and easy propagation (US Department of Agriculture, 2020). Vaseygrass is a weed in several countries, such as New Zealand, the United States, Australia, South Korea, Japan, India, Puerto Rico, and Argentina. In the United States, this plant was introduced as a candidate forage grass, but due to its low palatability and high seed production, it was not used and became a weed in pastures, especially in Florida (Lee et al., 2013; Pereira et al., 2017).

Vaseygrass can be controlled by physical, mechanical, biological, and chemical methods. In areas with localized infestation, continuous grazing can reduce the weed's canopy by 15%, favoring the competition by other forage species (Newman, Sollenberger, 2005). It is important to highlight that the control of Poaceae plants in pasture areas is difficult, due to the botanical similarities among forage and non-forage grass, so the methods must be chosen carefully. In this sense, Jeffries et al. (2017) observed that the application of clethodim supplemented with grass cutting improved the control of vaseygrass, but not with imazapic and metsulfuron + nicosulfuron. Studies have been conducted on the use of glyphosate for vaseygrass control, but none with the mixture of glyphosate + nicosulfuron (González-Ibáñez, 1987; Newman and Sollenberger, 2005).

The herbicides imazapic and nicosulfuron belong to the same mechanism of action, the inhibition of acetolactate synthase (ALS), while glyphosate inhibits the enzyme EPSPS (5-enolpyruvylshikimate 3-phosphate synthase). In Brazil, resistance to imazapic or glyphosate has been reported in *Amaranthus palmeri*, *Amaranthus hybridus*, and *Lolium* spp. (Netto et al., 2016; Collavo, Sattin, 2014).

This demonstrates that the history of weed management in an area can influence weed response to herbicides and, over time, increase resistance evolution (Duke, Powles, 2008). In vaseygrass management, chemical and mechanical control are frequently used by producers (Jeffries et al., 2017); however, information regarding chemical control of this species is still scarce. Therefore, there is a need to understand both the physiological response of vaseygrass to herbicides and the tolerance of populations originating from areas with different management histories.

The genus *Paspalum* exhibits enormous genetic diversity: approximately 75% of its 350 known species are polyploid, and most of these are apomictic polyploids (Galdeano et al., 2016). Ploidy levels range from diploid to octoploid, with tetraploidy being the most frequent condition. Additionally, interspecific hybrids derived from diploid cytotypes of *P. stellatum*, *P. malmeanum*, and *P. schesslii* can give rise to allopolyploid complexes (Bonasora et al., 2018). In many *Paspalum* species, ploidy level often correlates with reproductive mode, with diploids generally being sexual and tetraploids typically apomictic. This can influence herbicide tolerance through gene dosage effects. Determining ploidy level is therefore important in studies with distinct accessions and populations, as herbicide efficacy varies with ploidy – documented in *Rottboellia cochinchinensis* (Alves et al., 2003) and *Lolium perenne*, where tetraploid genotypes were more tolerant to glyphosate than diploids (Dors et al., 2010). Despite this, the ploidy of vaseygrass remains poorly studied, and its implications for weed management are still unexplored.

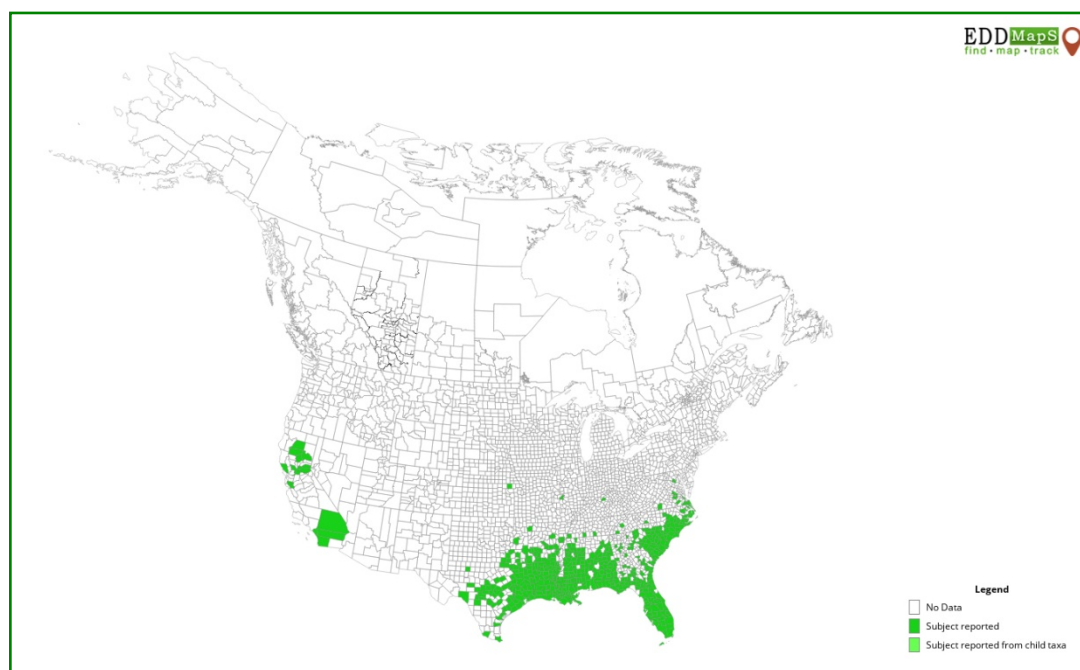
Beyond genetic diversity, the differences in herbicide history and management practices can drive vaseygrass populations toward distinct resistance mechanisms and physiological states. Some populations may evolve specific target-site mutations, others may develop broad metabolic tolerance with complex stress-response and epigenetic signatures, and many will exhibit variation in growth and competitive traits as part of this adaptive process (Jeffries et al., 2017; Comont et al., 2020).

Thus, with the hypothesis that distinct populations can present differences in chemical control sensitivity as a result of different selection pressures provided by distinct management methods, this study aimed to determine the ploidy level and evaluate chemical control of two populations of vaseygrass historically exposed to herbicide selection pressure.

2. Material and methods

2.1 Plant material collection

Vaseygrass (*P. urvillei*) seeds were collected from two locations in Alachua County, Florida, USA (Figure 1). This grass was introduced to the USA as pasture grass in the late 1800's. The earliest voucher specimen from Florida dates to 1943 (Florida Natural Areas Inventory – FNAI). The populations selected for seed collection have undergone different histories for weed management: i) Population 1 (hereafter referred to as *Fence*): is comprised of plants growing in proximity to a *Fence* and exposed



Source: EDD Maps <https://www.eddmaps.org/species/subject.cfm?sub=6152>

Figure 1 - Distribution map of vaseygrass (*Paspalum urvillei* Steud.) in the United States (2024)

only to chemical control with glyphosate (29°35'35"S and 82°35'42"O); and ii) Population 2 (hereafter referred as *Pasture*): subjected to regular pasture mowing combined with herbicide application (29°78' 56"S and 82°41'47"O).

In both locations, seeds were collected using the same protocol. For each population, 60 individual vaseygrass plants were randomly selected. One inflorescence per plant was cut close to the base of the panicle, and each inflorescence was stored in a separate bag, yielding 60 individual samples per population. The samples were brought to the Forage Breeding and Genetics laboratory in Gainesville, FL, where the inflorescences were individually threshed and cleaned.

2.2 Ploidy level assessment

After seed cleaning, all clean seeds were mixed into one composite sample to represent each locality; no treatments were used to induce germination. Five seeds were sown per cell in plastic trays with 72 cells, with commercial substrate, and placed in the greenhouse (temperature 28 °C ± 2, and relative humidity 60%) for 25 days. After emergence, seedlings that showed the best vigor and were disease-free were transplanted into pots (1.2-L volume) filled with commercial substrate (composed of Canadian *Sphagnum* peat moss (85%), perlite, vermiculite, dolomitic and calcitic limestone, and wetting agent) and were irrigated daily. During the whole experiment, the plants were kept in the greenhouse where each pot received 30 mL of liquid fertilizer Miracle-Gro® (nitrogen 24%, phosphate 8%, potash 16%, boron 0.02%, copper 0.07%, iron 0.15%, manganese 0.05%, molybdenum 0.0005%, and zinc 0.06%) every 3 days.

For flow cytometry analysis, the experimental design was completely randomized. For each of the two vaseygrass populations. Approximately 100 mg of vaseygrass leaves at the vegetative stage were used. The leaves were sectioned into 1.0 mm fragments and placed in Petri dishes with 500 µL of a commercial extraction solution (Rios et al., 2015; CyStain UV Precise P, Sysmex®). The leaf tissue was chopped and incubated in the solution for 30 s. The samples were then filtered through a 50 µm CellTrics filter (Partec®; model: CellTrics 50 µm; Sysmex Corporation, Kobe, Japan). After filtration, 2 mL of staining solution containing propidium iodide and RNase (CyStain PI Absolute P, Sysmex®; Sysmex Corporation, Kobe, Japan) was added to stain the released nuclei. The samples were kept refrigerated in a polystyrene box with ice for 5 min and analyzed immediately on a BD Accuri C6 flow cytometer (Accuri® Cytometers; model: BD Accuri C6; BD Biosciences, San Jose, CA, USA) using the FL2 channel.

For each sample, at least 5,000 nuclei were counted and analyzed using the BD Accuri CFlow software (version 1.0.264.21; BD Biosciences, San Jose, CA, USA). Samples with histograms showing peaks with coefficients of variation less than 10% were considered for analysis. As controls, we

used the diploid and tetraploid *Paspalum notatum* Flügge cultivars: "Pensacola" (2n = 2x = 20) and "Argentine" (2n = 4x = 40). Additionally, 'B73' maize (*Zea mays*) was used to estimate genome size in vaseygrass. To evaluate ploidy level and estimate genome size, propidium iodide (PI) was used as a DNA marker. The PI in solution binds to DNA and fluoresces when excited by an energy source. The amount of PI bound to DNA is proportional to the DNA content in the sample; therefore, the fluorescence intensity recorded by the flow cytometer is an accurate estimator of DNA content per cell. The flow cytometer recorded the fluorescence intensity of PI bound to DNA from cells in the G1 and G2 phases of interphase. Consequently, the DNA content of diploid nuclei (2C DNA content) was calculated (equation 1) based on the fluorescence intensity recorded for the G1 peak (Rios et al., 2015).

Equation 1

$$2C \text{ DNA content} = \frac{\text{mean position G1 vaseygrass peak} \times \text{standart DNA content}}{\text{mean position G1 standart peak}}$$

Where,

2C DNA content = Nuclear DNA content in picograms (pg) of the unknown sample (vaseygrass). Mean position G1 vaseygrass peak = Position of the G1 peak obtained for the vaseygrass sample. Standard DNA content = Known DNA content (in pg) of the internal standard. Mean position G1 standart peak = Position of the G1 peak obtained for the internal standard.

2.3 Effectiveness of chemical control

The experiment was conducted in a greenhouse at the Forage Breeding and Genetics Laboratory, Gainesville, Florida, USA. The pots were filled with a commercial substrate, as described in the previous section. When the seedlings developed the first true leaf, they were transplanted into 300-mL pots (approximately 25 d after sowing). The initial transplant into 300-mL pots was performed to facilitate seedling establishment and minimize stress; after 35 days, when the plants had four true leaves, they were moved to 0.5-L pots for the remainder of the experiment. After the acclimatization period (approximately 20d after transplanting), at V4 stage, the plants were subjected to herbicide treatments.

The experimental design was a randomized complete block in a 2 x 5 factorial treatment arrangement with four replicates. The first factor was the vaseygrass population with two levels (*Fence* and *Pasture*). The second factor was the herbicide treatment with five levels: T1 – Control, T2 – imazapic, 70 g ha⁻¹ (Cadre® 0.29 L ha⁻¹), T3 – nicosulfuron, 750 g ha⁻¹ (Accent® 170.24 g ha⁻¹), T4 – glyphosate, 356 g ha⁻¹ (Sunphosate® 1.16 L ha⁻¹), and T5 – nicosulfuron, 750 g ha⁻¹ (Accent® 170.24 g ha⁻¹), + glyphosate, 356 g ha⁻¹ (Sunphosate 1.16 L ha⁻¹). The

doses were based on field use rates. A non-ionic adjuvant (Induce®) was added to all treatments at 0.5% v/v. The experiment was performed twice.

Herbicide applications were conducted in a CO₂-pressurized chamber sprayer fitted with a single TeeJet TT 11005 flat-fan nozzle (TeeJet Technologies, Springfield, IL, USA). The chamber sprayer was calibrated to deliver 187 L ha⁻¹ at 2.8 bars. At 7, 14 and 21 days after treatment application (DAT), herbicide efficacy was evaluated visually using a scale adapted from the European Weed Research Council (1964), as follows: 1- Null; 2- Very light; 3- Light; 4- Regular; 5 - Average; 6- Almost strong; 7- Strong; 8-Very strong; 9- Extremely strong; 10- Total destruction. Concomitant to these assessments, photos of phytotoxicity symptoms were collected. Plant height (cm) and culm diameter (mm) were measured; number of leaves, and number of tillers were counted; and the SPAD index (Soil Plant Analysis Development) was measured on the third leaf. At 21 DAT, shoots were cut and placed in an oven with air circulation at a temperature of 45°C for 96 h, to determine dry matter production.

2.4 Statistical analysis

The data were subjected to normality analysis of the residuals by the Shapiro-Wilk test at 5% probability and the Bartlett test at 5% probability to verify the homogeneity of variance. The plant culm diameter and number of leaves showed non-normal variance distribution and were transformed using the Box-Cox method. The homogeneity of variances for all variables was verified after transformation. Weed control data were transformed using the square root, $x + 0.5$. The model used was $y = \text{population} * \text{herbicide} + \text{block} + \text{control} * \text{trial}$, herbicide was the treatment; control is the non-treatment;

and the trial is an experiment. The analysis of variance was performed on transformed data using the F test, and means were compared using Tukey's test ($\alpha = 0.05$), using the R Core Team software (R Core Team, 2019).

3. Results and discussion

3.1 Ploidy level assessment

The ploidy analysis revealed that both populations had similar DNA content and were classified as tetraploid ($2n = 4x = 40$) based on the comparison of their G1 peaks and those from the tetraploid bahiagrass, "Argentine" ($2n = 4x = 40$) (Figure 2). The results were consistent across two runs. The average DNA content was 2.31 pg for the *Fence* population and 2.25 pg for the *Pasture* population.

Flow cytometry allows rapid and accurate determination of ploidy level in vaseygrass genotypes collected from *Pastures*. Knowing the ploidy level matters because it is often associated with reproductive mode in *Paspalum* species: diploids are typically sexual, while tetraploids are usually apomictic, and ploidy can also affect herbicide tolerance and invasive potential. Among the *Paspalum* species studied from South America, Vaio et al. (2007) reported 2C values between 2.29 pg and 2.43 pg for *Paspalum urvillei* and 2.40 pg for genotypes in Brazil, indicating that the species is consistently tetraploid across its range. However, we confirmed the ploidy of the populations used in this study to rule out any possible local cytotypic variation that might affect response to herbicides. The authors found plants to be tetraploid, as reported in this current study. Burson (1979) studied the crosses of several species in the genus *Paspalum* and found that *P. urvillei* has $2n = 40$, corroborating the level of ploidy found in the populations in this study.

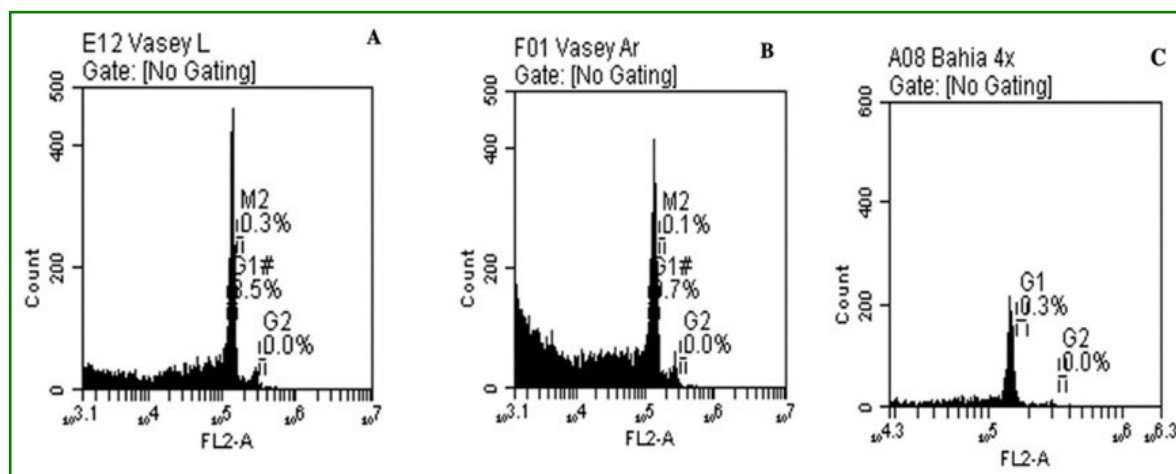


Figure 2 - Histograms of the fluorescence intensity of DNA in cell nuclei extracted from leaves of (A) Population of vaseygrass from *Fence* area, (B) Population of vaseygrass from *Pasture* area and (C) Population of *Paspalum notatum* Flugge (tetraploids). Parodi cv. Argentine

Knowledge of ploidy level in *Paspalum* species is important for designing effective weed management strategies, as ploidy influences both reproductive mode and herbicide tolerance. When polyploids are apomictic – which is common in several perennial grass genera like *Paspalum* – any herbicide tolerant genotype becomes fixed and is transmitted clonally to all offspring. Moreover, polyploid plants can exhibit higher baseline tolerance to herbicides due to gene dosage effects. Ploidy differences also enhance understanding of evolutionary processes: polyploidy provides genetic redundancy that may buffer against deleterious mutations, and allopolyploidy (resulting from interspecific hybridization) can combine resistance traits from different species, accelerating the evolution of herbicide resistance (Galdeano et al., 2016; Rutland et al. 2021). Bobadilla et al. (2021) reported that tetraploid Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum*) plants exhibited higher tolerance to glyphosate than diploid plants of the same subspecies and suggested that understanding the mechanisms behind this ploidy dependent tolerance could help design strategies to slow the spread of herbicide resistance. Alves et al. (2003) identified ploidy variation in itchgrass (*Rottboellia cochinchinensis*) accessions (one diploid, others polyploid) but did not assess differential response to herbicides. Thus, while ploidy may be associated with weedy traits, causal relationship has not been established for this species.

For *Lolium perenne*, Dors et al. (2010) reported that a tetraploid Italian ryegrass genotype required 1.6 times the glyphosate dose to achieve the same level of control as that of a diploid genotype, suggesting that ploidy can influence herbicide efficacy. However, other studies have found no difference in glyphosate efficacy across ploidy levels,

indicating that ploidy-related tolerance is not universal and depends on genetic background and environmental factors. Therefore, diploid plants may be more susceptible to glyphosate than tetraploids.

3.2 Effectiveness of chemical control

The test for chemical control allowed us to observe the response of the two populations to different chemical treatments. Significant ($p < 0.05$) two-way interactions were observed for plant height between population and treatments (7 DAT and 14 DAT). At 7 DAT, the *Fence* population was more adversely affected by nicosulfuron and glyphosate than the *Pasture* population. At 14 DAT, the *Pasture* population exhibited a greater reduction in plant height following imazapic application than the *Fence* population, whereas the responses to the remaining herbicides were similar between populations (Table 1). At 21 DAT, all herbicides reduced plant height in both populations relative to the control ($p < 0.001$), and the interaction was no longer significant (Table 1).

For both populations, the number of leaves was lower after treatment with glyphosate, compared with nicosulfuron and imazapic at 7 DAT (Table 1). At 14 and 21 DAT, differences between the *Fence* and *Pasture* populations were significant as plants from the former area had more leaves than the latter. All herbicide treatments significantly reduced the number of leaves.

The effectiveness of herbicides in reducing plant height and leaf area is highly desirable in weed management, as it can create a competitive advantage for forage plants over weeds (Table 1). Glyphosate is a nonselective, post-emergence herbicide widely used in vegetation

Table 1 - Interaction effects of population and herbicide treatments on plant height (PH) and Number of leaves (NPL) measured at 7, 14 and 21 days after application of treatments on *Paspalum urvillei* Steud (vaseygrass)

Treatments	Pop	PH (cm) 7	PH (cm) 14	PH (cm) 21	NPL 7	NPL 14	NPL 21
Control	<i>Fence</i>	13.40aA	17.2aA	20.19aA	8.00aAB	13.00bA	17.0bA
	<i>Pasture</i>	12.20aAB	15.6aA	16.98aA	8.00aAB	19.00aA	23.5aA
Imazapic	<i>Fence</i>	9.82aA	8.25aB	4.05aB	8.25aA	7.25aB	1.5aC
	<i>Pasture</i>	10.5aAB	8.48aA	3.59aB	8.25aA	7.75aB	1.0aBC
Nicosulfuron	<i>Fence</i>	7.91bA	7.90aB	5.55aB	9.00aA	9.50aB	6.50aB
	<i>Pasture</i>	15.7aA	12.6aA	4.81aB	9.00aA	10.0aB	4.75aB
Glyphosate	<i>Fence</i>	9.42aA	8.70bAB	4.83aB	5.00aB	7.5aB	0.0aC
	<i>Pasture</i>	7.78aB	17.69aA	6.02aB	5.00aB	7.5aB	2.0aBC
Nicosulfuron + Glyphosate	<i>Fence</i>	8.21aA	6.18aB	3.85aB	6.66aAB	9.00aB	0aC
	<i>Pasture</i>	10.3aAB	10.6aA	3.88aB	6.66aAB	8.33aB	0aC
p-value		0.017*	0.21*	0.0001*	1.00	0.013*	0.023*

The numbers are means of two experiments and four replications. The same lowercase letters in the column mean that the populations do not differ from each other based on Tukey's test ($p > 0.05$). Capital letters in the column indicate that the herbicide treatments do not differ based on Tukey's test ($p > 0.05$)

* Significant P-value > F

desiccation and *Pasture* renovation. In general, symptoms (generally chlorosis) start to manifest in the first week after application, followed by leaf necrosis (Duke, Powles, 2008). Glyphosate controlled both populations 100%.

The number of tillers at 7 DAT, significantly differed between populations ($p < 0.05$) in response to glyphosate and nicosulfuron + glyphosate treatments. For both treatments, the plants from *Fence* had less tillers than those from *Pasture* (Table 2). At 14 DAT, these differences remained for glyphosate, as it killed all tillers of *Fence* plants. At 21 DAT, all plants from both locations had less tillers compared to their respective nontreated checks.

In general, all Poaceae weed species are difficult to control in pasture areas. The capacity to perpetuate is because of high tillering in a short period. Therefore, quantification of tiller numbers is important for strategic control. Herbicides that affect the tillers can reduce regrowth, especially for grasses with bunch-type growth habit. All treatments tested reduced the number of tillers, which means that the herbicides reduced the energy reserves allocated to tiller formation.

The plant culm diameter (Table 2) decreased after the application of herbicides, as a result of physiological impairment such as photosynthesis reduction. The same pattern was observed for the other variables previously mentioned (Table 1). At 21 DAT, all treatments were different from the nontreated check, regardless of population. For nicosulfuron + glyphosate treatment, *Fence* plants had a smaller culm diameter compared to *Pasture*. In general, culm diameter decreased after the application of herbicides (Table 2).

The SPAD index (Table 3), together with the other variables described above, helps understand plant responses to the applied herbicides. Herbicides belonging to HRAC Groups 2 (nicosulfuron) and 9 (glyphosate) inhibit amino acid biosynthesis, leading to protein starvation, chlorophyll degradation, and ultimately a decline in SPAD values. The low SPAD index recorded for treated plants, therefore, reflects herbicide-induced leaf chlorosis and photosynthetic impairment. At 7 DAT, there was no difference between the control and herbicide treatments, except with nicosulfuron. At 14 DAT, the differences between glyphosate, nicosulfuron + glyphosate, and imazapic were noted for both populations. At 21 DAT, as herbicide action progressed, there was a difference between all treatments and the control, since the plants of both populations had symptoms of phytotoxic effects, such as loss of photosynthetically active tissue. The SPAD index, which provides a rapid estimate of leaf chlorophyll content, can detect genetic variability between accessions because chlorophyll content is a heritable quantitative trait. Significant differences in SPAD values *Fence* and *Pasture* reflect underlying genetic differences, as observed in several grass species (Oliveira et al., 2020; Baek et al., 2025). However, as recommended by Oliveira et al. (2020), SPAD data should be used in combination with other traits (such as plant height) to provide a robust assessment of genetic variability.

Previous studies reported SPAD index values for healthy *P. notatum* plants between 37.67 and 39.80, while *P. regnellii*, *P. conspersum*, and *P. malacophyllum*,

Table 2 - Interaction effects of population and herbicide treatments on Number of Tillers (NPT) and Plant culm diameter (PSD) at 7, 14 and 21 days after application of treatments on *Paspalum urvillei* Steud (vaseygrass)

Treatments	Pop	NPT 7	NPT 14	NPT 21	PSD (mm) 7	PSD (mm) 14	PSD (mm) 21
Control	<i>Fence</i>	1.33aA	4.00aA	6.00aA	3.36bA	4.77aA	6.12aA
	<i>Pasture</i>	2.00aA	3.33aA	5.00bA	5.32aA	5.93aA	4.60aA
Imazapic	<i>Fence</i>	1.0aA	0.5aBC	0.00aB	3.87aA	2.40aBC	1.57aBC
	<i>Pasture</i>	1.5aA	1.0aBC	0.25aB	3.55aA	2.05aB	0.85aB
Nicosulfuron	<i>Fence</i>	1.50aA	1.25aB	0.75aB	3.63aA	4.60aAB	2.5aB
	<i>Pasture</i>	1.75aA	1.75aB	0.50aB	4.50aA	3.45aAB	2.6aB
Glyphosate	<i>Fence</i>	0.33bA	0.00bC	0.00aB	3.77aA	2.10aABC	1.6aBC
	<i>Pasture</i>	2.00aA	1.5aB	0.00aB	4.07aA	2.82aB	1.7aB
Nicosulfuron + Glyphosate	<i>Fence</i>	0.5bA	0.25aBC	0.00aB	3.05aA	2.02aC	0.52bC
	<i>Pasture</i>	1.5aA	0.00aC	0.00aB	3.13aA	2.38aB	1.17aB
p-value		0.1512	0.013*	0.1618	0.41	0.59	0.43

The numbers are means of two experiments and four replications. The same lowercase letters in the column mean that the populations do not differ from each other based on Tukey's test ($p > 0.05$). Capital letters in the column indicate that the herbicide treatments do not differ based on Tukey's test ($p > 0.05$)

* Significant P-value > F

Table 3 - Interaction effects of population and herbicide treatments on SPAD and Visual phytotoxicity (VP) at 7, 14, and 21 days after application of treatments on *Paspalum urvillei* Steud (vaseygrass)

Treatments	Pop	SPAD 7	SPAD 14	SPAD 21	VP 7	VP 14	VP 21
Control	Fence	43.55aA	35.70aA	34.70aA	1.00aD	1.00aE	1.00aC
	Pasture	36.17aA	32.45aA	31.90aA	1.00aD	1.00aE	1.00aC
Imazapic	Fence	34.40aAB	24.92aAB	1.00aB	4.25aBC	7.00aC	9.75aA
	Pasture	28.70bA	16.50aBC	3.72aB	4.25aB	7.00aC	9.75aA
Nicosulfuron	Fence	23.72aB	30.80aA	8.95aB	3.75aC	6.00aD	8.50aB
	Pasture	40.55aA	29.10aAB	6.35aB	3.33aB	6.00aD	8.75aB
Glyphosate	Fence	34.07aAB	9.90aB	1.00aB	5.5aAB	8.00aB	10.0aA
	Pasture	27.30aA	13.35aBC	1.00aB	5.5aA	8.00aB	10.0aA
Nicosulfuron + Glyphosate	Fence	27.45aAB	10.67aB	1.00aB	6.5aA	9.00aA	10.0aA
	Pasture	26.35aA	7.75aC	1.00aB	6.5aA	9.00aA	10.0aA
p-value		0.07	0.52	0.47	0.93	1.00	0.91

The numbers are means of two experiments and four replications. The same lowercase letters in the column mean that the populations do not differ from each other based on Tukey's test ($p > 0.05$). Capital letters in the column indicate that the herbicide treatments do not differ based on Tukey's test ($p > 0.05$).

* Significant P-value > F

have SPAD values of 34.16, 34.48, and 33.5, respectively (Pereira et al., 2017; Beloni et al., 2017). These differences show that, under similar growing conditions, *P. notatum* had a leaf chlorophyll content of 37.67, whereas the other three species had lower values, suggesting interspecific genetic variation in chlorophyll biosynthesis or photosynthetic capacity. However, SPAD differences alone do not directly indicate forage quality or stress tolerance, as these traits are influenced by multiple factors.

Nevertheless, the low SPAD values observed at 21 DAT for plants after herbicide application may be related to herbicide induced oxidative stress. Oxidative stress induces lipid peroxidation of unsaturated fatty acids in thylakoid membranes, disrupting membrane integrity (Gomes, Juneau, 2016; Freitas Silva et al., 2017). This disruption leads to chlorophyll degradation – through both enzymatic breakdown and photo oxidation – because chlorophyll protein complexes are no longer stabilized within the damaged thylakoids. Since the SPAD index measures relative leaf chlorophyll content, chlorophyll degradation results in lower SPAD values. In addition, oxidative damage influences other essential metabolic processes, including changes in stomatal aperture and reduced photosynthetic capacity (Gomes, Juneau, 2016; Freitas Silva et al., 2017).

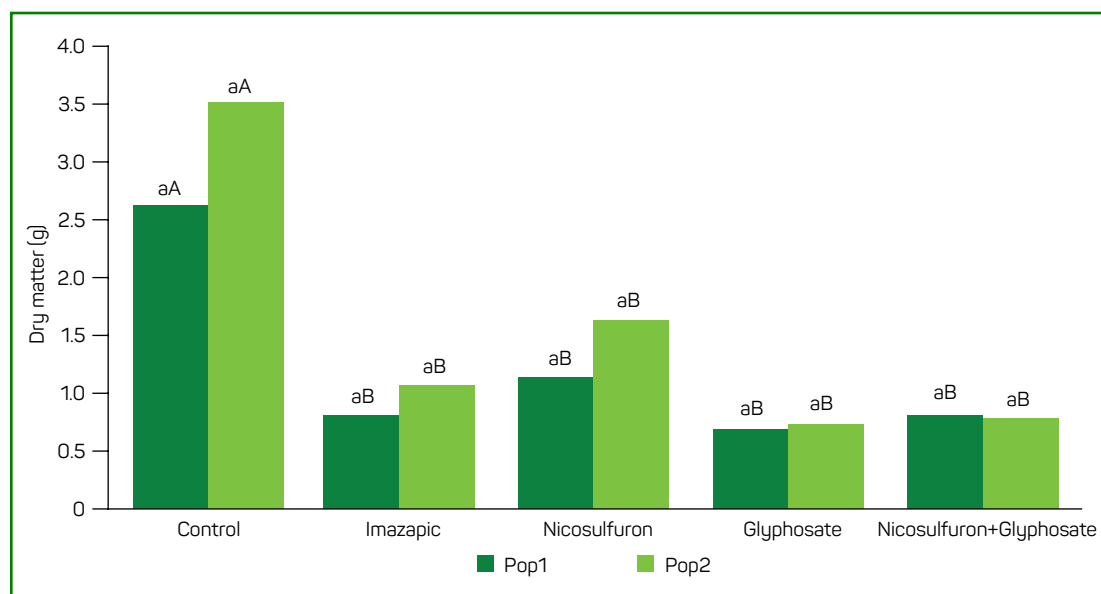
The scores for visible plant injury showed that glyphosate and nicosulfuron + glyphosate are highly phytotoxic to both populations at 7 and 14 DAT (Table 3); however, the effect of nicosulfuron was less than that of the other herbicides. Although nicosulfuron resulted in 87% control of vaseygrass, this was lower when compared to the other herbicides tested here. For dry matter, significant

differences ($p < 0.05$) were observed between herbicide treatments (Figure 3) but not between populations. All herbicides reduced dry biomass by approximately 74% compared to the non-treated check.

In similar studies, Davies and Hamerlynck (2019) reported that imazapic reduced the ground cover and plant density of the exotic grass *Ventenata dubia*, attaining 82% to 94% control, which is considered satisfactory. In this current study, imazapic controlled vaseygrass 97% (Table 3).

Glyphosate can be recommended to control vaseygrass in Florida pastures, as it can be applied alone or in a tank mixture, resulting in 100% control. Glyphosate can be used for pasture restoration (Mello et al., 2016). It is important to use the correct herbicide and optimum dose because populations most likely harbor differential tolerance to suboptimal doses as influenced by localized adaptations to stress factors (Belz and Cedergreen, 2010), plant growth stage (Velini et al., 2008), and time after herbicide application (Belz and Duke, 2014).

From the current perspective, it is essential to interpret data from these studies with caution. Furthermore, plant responses to glyphosate – as well as to other herbicides – may vary with rising atmospheric CO₂ concentrations. Elevated CO₂ is now a relevant factor in pastures and other agricultural systems, where it has been shown to alter weed physiology and herbicide efficacy (Fernando et al., 2016). C3 weeds are more responsive to elevated atmospheric CO₂ than C4 weeds, which may alter selection pressure for herbicide resistance, although direct evidence linking C3 metabolism to the evolution of resistance is limited (Fernando et al., 2016).



* Significant P-value > F

Figure 3 - Average values of *Paspalum urvillei* Steud (vaseygrass) dry matter (g) at 21 days after application of treatments. The numbers are means of two experiments and four replications. The same lowercase letters in the column mean that the populations do not differ from each other based on Tukey's test ($p > 0.05$). Capital letters in the column indicate that the herbicide treatments do not differ based on Tukey's test ($p > 0.05$)

4. Conclusions

Both populations of *Paspalum urvillei* are tetraploid. Glyphosate alone or mixed with nicosulfuron controls 100% of vaseygrass plants, regardless of populations and, therefore, are the best options to manage this pasture weed. Imazapic controls 90% of vaseygrass and can be used in conjunction with a mechanical weed control method to allow excellent growth of desired forage grasses.

Author Contributions

All authors read and agreed to the published version of the manuscript. RTSS, PLCAA, and EFR: conceptualization of the manuscript and development of the methodology. RTSS, and EFR: data collection and curation. RTSS, and ACMP: data analysis. RTSS, PLCAA, EFR, and ACMP: data interpretation. RTSS, PLCAA, and EFR: funding acquisition and resources. RTSS, PLCAA, and EFR: project administration. EFR: supervision. RTSS, and ALB: writing

the original draft of the manuscript. RTSS, PLCAA, and ALB: writing, review and editing.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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