Ecophysiological response of *Sorghum halepense* populations to reduced rates of nicosulfuron

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Abstract – The control and regrowth after nicosulfuron reduced rate treatment of Johnsongrass (*Sorghum halepense* L. Pers.) populations, from seven Argentinean locations, were evaluated in pot experiments to assess if differential performance could limit the design and implementation of integrated weed management programs. Populations from humid regions registered a higher sensibility to reduced rates of nicosulfuron than populations from subhumid regions. This effect was visualised in the values of regression coefficient of the non-linear models (relating fresh weight to nicosulfuron rate), and in the time needed to obtain a 50% reduction of photosynthesis rate and stomatal conductance. The least leaf CO_2 exchange of subhumid populations could result in a lower foliar absorption and translocation of nicosulfuron, thus producing less control and increasing their ability to sprout and produce new aerial biomass. The three populations from subhumid regions, with less sensibility to nicosulfuron rates, presented substantial difference in fresh weight, total rhizome length and number of rhizome nodes, when they were evaluated 20 week after treatment. In consequence, a substantial Johnsongrass re-infestation could occur, if rates below one-half of nicosulfuron labeled rate were used to control Johnsongrass in subhumid regions.

Index terms: Johnsongrass, weed control, weed regrowth.

Resposta ecofisiológica de populações de *Sorghum halepense* a doses reduzidas de nicosulfuron

Resumo – Foram conduzidos experimentos sob condições controladas, a fim de se investigar os efeitos de doses reduzidas de nicosulfuron, no controle e rebrota de populações de capim massambará (*Sorghum halepense*), de sete localidades da Argentina, visando programas de manejo integrado. As populações das regiões úmidas registraram maior sensibilidade a doses reduzidas de nicosulfuron, em relação àquelas das regiões subúmidas. Esse efeito foi observado nos valores do coeficiente de regressão, das curvas de dose-resposta de modelo log-logístico, que relacionam a biomassa fresca com doses de nicosulfuron; tal efeito foi observado também no tempo necessário para se obter 50% da redução da fotossíntese e da condutância estomática. O menor intercâmbio de CO_2, obtido nessas populações subúmidas, pode ter contribuído para a absorção e translocação mais baixa do nicosulfuron, tendo produzido menor controle e, conseqüentemente, tendo aumentado sua capacidade de rebrotar e produzir nova biomassa aérea ao final do ciclo. As três populações das regiões subúmidas, com menor sensibilidade ao nicosulfuron, apresentaram uma diferença substancial na biomassa fresca, no comprimento total e no número de nódulos dos rizomas, quando foram avaliadas vinte semanas após o tratamento com herbicida. Os resultados obtidos sugerem que se forem usadas doses de nicosulfuron menores do que as recomendadas, pode ocorrer uma severa rebrota do capim massambará em regiões subúmidas.

Termos para indexação: capim massambará, controle de planta daninha, capacidade de rebrota.

Introduction

Johnsongrass (*Sorghum halepense* L. Pers.), an aggressive perennial grass, is considered one of the world's worst weeds (Holm et al., 1977) and has proved troublesome in the extensive cropping systems of Argentina (Leguizamón, 1999), despite continued selective postemergence herbicide control.

The efforts to reduce herbicide use, for both environmental and economic reasons (Buhler, 1999), have promoted the development of integrated weed management program.

Studies based on a Johnsongrass population indicates that reduced rates of herbicides of both nicosulfuron and clethodim provide an adequate control of that weed (Jordan et al., 1996; Rosales Robles et al., 1999a, 2001).

However, the effectiveness of this technology has been related to several factors such as weed spectrum, environmental conditions, weed populations and herbicide mode of action (Doyle et al., 2001). The problem is that weeds tend to adapt quickly to change, and produce a wide variety of genotypes that can be fit to a range of environments (Buhler, 1999).

Despite the huge knowledge on Johnsongrass ecophysiology (Mc Whorter, 1972; Mc Whorter & Jordan, 1976; Stuart et al., 1985), little work has focused on the ecophysiological response of Johnsongrass populations, when treated with reduced rates of herbicides. Possible differential performance of populations could limit the design and implementation of integrated weed management programs, in different crop production systems.

The objective of this study was to evaluate the ecophysiological response of seven Johnsongrass populations, from different Argentinean locations, when reduced rates of nicosulfuron were employed, and to determine whether differential performance could modify population recovery from herbicide treatment.

Material and Methods

Rhizomes of Johnsongrass populations were originally collected in April 1999 from corn (*Zea mays*) fields of seven Argentinean locations (Table 1). Rhizomes from all locations were stored at 4°C in the fall and winter of that year. The rhizomes were grown in 50-L pots in October 1999. These materials grew vigorously, and provided a supply of new rhizomes that were used for experiments in the spring 2000.

Rhizomes of Johnsongrass were trimmed off into pieces with two nodes each, weighting from 4 to 7 g. These pieces were washed-free of soil, then soaked in a solution of 0.35 g L^{-1} benomyl for 10 min to retard decay. Rhizomes were layered in trays filled with sand, and held in growth chamber up to experiments were established. Temperature and relative humidity were 25°C and 65%, respectively.

Two outdoor experiments with pots were conducted in 2000 and 2001, at La Plata National University Station, 34°S, 57°W, La Plata, Argentina. Johnsongrass populations were grown from sprouted rhizomes in 30-L pots filled with a mixture (1:1) of soil and vermiculite. The samples were planted on October 10, 2000 and October 6, 2001. Plants were thinned to two per pot, four days after emergence. Pots were fertilized at planting with the equivalent of 100 kg ha^{-1} commercial granules fertilizer (18–46–0, N–P–K). The ecotypes were watered every day with a calculated amount of water during weed growing cycle to simulate rainfall regime of each location. The experimental design was a randomized complete block factorial over time with five replications. Factors evaluated were herbicide rates and weed populations.

Johnsongrass plants, with three to four fully expanded leaves (leaf collar visible), were sprayed with nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl) amino]carbonyl]amino] sulfonyl]-N,N-dimethyl-3-pyridinecarboxamide), by November 8, 2000, and by November 12, 2001. Rates for herbicide were 0X (untreated control), 0.125X, 0.25X, 0.5X, 0.75X, 1.0X and 1.5X of the labeled rate (1.0X, 35 g ha^{-1} a.i.). A nonionic surfactant at 0.25% (V/V) was added to nicosulfuron. After nicosulfuron application, photosynthesis (µmol m^{-2} s^{-1}) and stomatal conductance for water vapour (mM H_2O m^{-2} s^{-1}) were measured, simultaneously, to determine if differences between populations exist in time needed to render Johnsongrass physiologically non-competitive.

According to Ferrell et al. (2003), Johnsongrass was no longer physiologically and competitive after photosynthesis, and was reduced to 50% of that control. The last fully expanded leaf of each population plant was used throughout the experiment for data registration. Daily measurements were carried out in the first 15 days after herbicide treatment.

Table 1. Details of Argentinean locations from where Johnsongrass populations were extracted.

Population source	Province	Latitude	Longitude	Climate	Average rainfall (mm $year^{-1}$)	Average temperature (°C)
Santa Rosa	La Pampa	36°37’S	64°17’W	Subhumid	621	15.0
General Pico	La Pampa	35°40’S	63°44’W	Subhumid	669	16.1
Río Cuarto	Córdoba	33°08’S	64°21’W	Subhumid	815	16.7
La Plata	Buenos Aires	34°55’S	57°57’W	Humid	1,023	16.3
Rojas	Buenos Aires	34°12’S	60°44’W	Humid	990	16.4
Paraná	Entre Ríos	31°44’S	60°32’W	Humid	1,016	18.0
Laboulaye	Córdoba	34°07’S	63°23’W	Subhumid	837	16.2

For each measurement, photosynthesis and stomatal conductance values were expressed as a percentage of the control not treated, to adjust for daily variations. A linear interpolation was used to obtain the time needed for 50% reduction in photosynthesis and stomatal conductance, for each herbicide rate and population, relatively to untreated pots (Ferrel et al., 2003). Johnsongrass plants were harvested four weeks after treatment (WAT), by December 10, 2000 and by December 11, 2001, and fresh aboveground biomass (g plant^{-1}) was registered. Estimates of Johnsongrass control (%) were based on fresh weight reduction compared to the untreated control.

Non-linear regression analysis techniques, similar to those employed by Chism et al. (1992), were used to fit the Johnsongrass fresh aboveground biomass curves, and to compare aboveground biomass in different populations. The following model was used:

$$y = a_1 + a_2 \exp(-a_3 H), \quad (1)$$

in which Y is the weed fresh aboveground biomass (g); a_1 is the lower asymptote fresh aboveground biomass (g); a_2 is the aboveground biomass reduction from upper to lower asymptote (g); a_3 is the herbicide rate at which lower aboveground biomass is obtained (established as 1/X where the labeled herbicide rate is 1.0X); and H is the herbicide concentration in X (Figure 1). According to Rosales Robles et al. (2001), a_1 and a_2 initial estimates were obtained from Figure 1, while initial value of a_3 was obtained by the model:

$$a_3 = [-\ln (y - a_1)/a_2]H^{-1}. \quad (2)$$

An approximate coefficient of determination (R^2) was calculated to assess goodness of fit for individual non-linear equations, by subtracting the ratio of the residual sums of squares (RSS) to the corrected total sums of squares (CTSS) from 1, i.e., $R^2 = 1 - RSS/CTSS$ (Carey et al., 1997). Because a general non-linear model was used to generate aboveground biomass curves for all populations, comparisons between regression coefficients could be made using techniques described by Chism et al. (1992), to establish significant differences between regression lines.

The fresh weight (g), total rhizome length (cm), number of rhizome nodes, and number of seed on a plant basis, after a growth period of 16 weeks (20 WAT, by April 5, 2000, and by April 15, 2001) were recorded, in order to evaluate Johnsongrass potential regrowth from herbicide application.

Since an analysis of variance showed no significant interactions across two years, all data in figures were pooled and were presented collectively. Analysis of variance was performed on the number of days required for 50% reduction in photosynthesis and stomatal conductance, and on population regrowth and means separated using Fisher's protected LSD test ($p<0.05$).

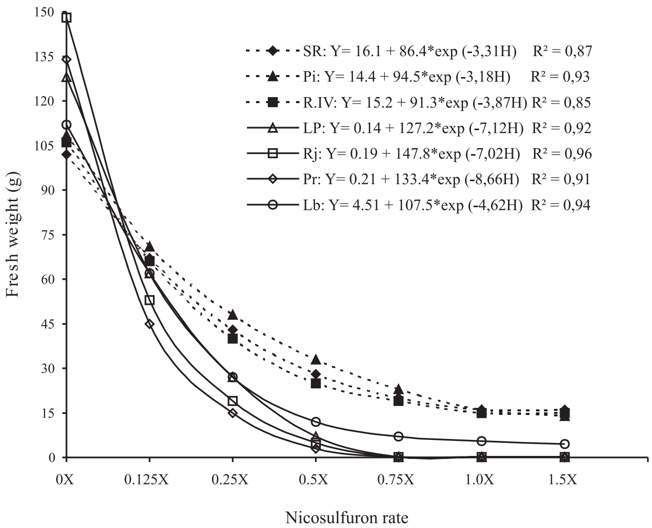

Figure 1. Effect of nicosulfuron reduced rates on fresh weight (g), of seven Johnsongrass populations, in Santa Rosa (SR), General Pico (Pi), Río Cuarto (R.IV), La Plata (LP), Rojas (Rj), Paraná (Pr) and Laboulaye (Lb), according to nonlinear regression equation: $Y = a_1 + a_2 \exp(-a_3 H)$.

Results and Discussion

The time of 50% reduction of photosynthesis rate and stomatal conductance for Santa Rosa, General Pico, and Río Cuarto populations was higher ($p<0.05$) than the one of La Plata, Rojas and Paraná populations. Laboulaye population showed an intermediate trend between both groups (Figures 1 and 2a). There was an increase in the time to obtain 50% reduction of both photosynthesis rate and stomatal conductance, in relation to reduced nicosulfuron rates. A significant ($p<0.05$) population x herbicide rate interaction was registered for the variables analysed (Figure 2).

Leaf gas exchange results showed considerable difference between the genotypes tested. Samples from humid populations registered a higher leaf gas exchange than those from subhumid ones. The capacity of the weed to colonise different areas was related to the large variability in growth and development responses of Johnsongrass (Monaghan, 1979).

According to results of this study, the lower leaf gas exchange obtained in the subhumid populations could favour the adaptability to subhumid and semiarid environments, while permitting the maintenance of the biotic potential of populations under less favourable environment. However, owing to this restricted leaf gas exchange, these populations from subhumid regions have been less susceptible to herbicide which is dependent upon distribution with current photosynthate. This is an important factor considering that nicosulfuron is symplastically translocated (Ahrens, 1994). Carey et al. (1997) have reported that tolerance of certain weeds to nicosulfuron has been related to different levels of absorption or translocation. These authors attributed the tolerance of eastern black nightshade (*Solanum ptycanthum*) to nicosulfuron to the low sensitivity of the acetolactate synthase (ALS) enzyme, and to the low translocation of the herbicide.

Comparison of regression coefficients revealed different population's responses to nicosulfuron reduced rates (Figure 1). The lower fresh weight asymptote (a_1), the difference between upper and lower weight asymptote (a_2), and the rate associated with lower weight asymptote (a_3), of aboveground biomass curves for Santa Rosa, General Pico, and Río Cuarto populations were different from those obtained in humid populations (Table 2 and Figure 1). Population from Laboulaye showed different regression coefficient from the other two groups. An adequate fit was observed in all populations with R^2 ranging from 0.85 to 0.96 (Table 2).

Johnsongrass control at four-six leaves stage with reduced rates of nicosulfuron was population related (Figure 3). Santa Rosa, General Pico and Río Cuarto populations' control was 35% (compared to fresh weight of untreated control) with nicosulfuron at 0.125X, while the control of populations from Rojas and Paraná was approximately 65%. La Plata and Laboulaye showed an intermediate control between the other two groups (Figure 3). Herbicide at 0.5X provided, approximately, 73% of Santa Rosa, Río Cuarto and General Pico populations control, while control for La Plata, Rojas, and Paraná populations were approximately 96%. The control of Laboulaye population was 89% with nicosulfuron at 0.5X (Figure 3). No further significant ($p<0.05$) aboveground biomass reduction was observed above 0.5X for La Plata, Rojas, Paraná and Laboulaye, and above 0.75X for Santa Rosa, General Pico and Río Cuarto. Plants from these three populations were less sensitive to nicosulfuron than plants originating from the La Plata, Rojas, Paraná and Laboulaye populations, for all the nicosulfuron rates evaluated (Figure 3).

Previous studies have established that reduced rates of nicosulfuron controlled rhizome Johnsongrass, effectively, when applied at early stages

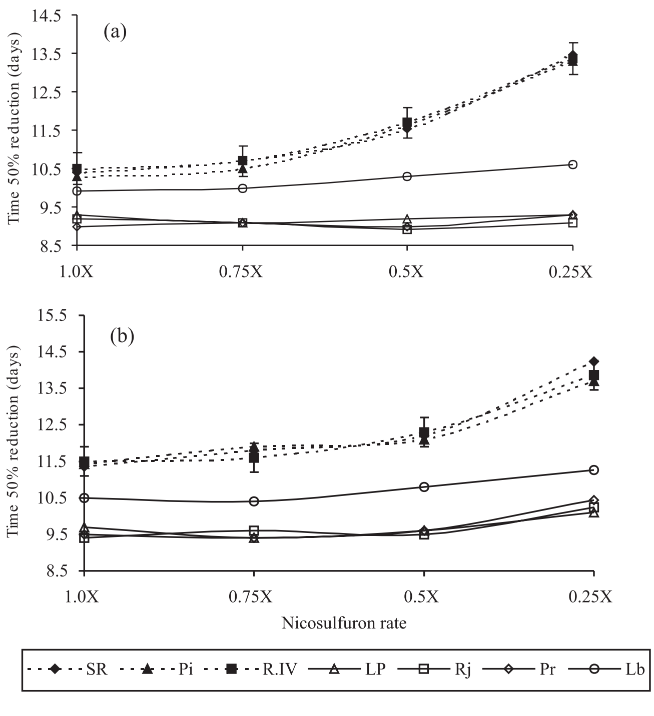

Figure 2. Number of days required for 50% reduction in photosynthesis (a), and stomatal conductance (b) of seven Johnsongrass populations, in Santa Rosa (SR), General Pico (Pi), Río Cuarto (R.IV), La Plata (LP), Rojas (Rj), Paraná (Pr), and Laboulaye (Lb). Vertical bars are LSDs ($p<0.05$).

Table 2. Nonlinear regression coefficients for nicosulfuron rate response models, from seven Johnsongrass population[(1)].

Population	Asymptotes[(2)]			R^2
	a_1 (g pl^{-1})	a_2 (g pl^{-1})	a_3 (1 X^{-1})	
Santa Rosa	16.1a	86.4a	3.31a	0.87
General Pico	14.4a	94.5a	3.18a	0.93
Río Cuarto	15.2a	91.3a	3.87a	0.85
La Plata	0.14b	127.2b	7.12b	0.92
Rojas	0.19b	147.8b	7.02b	0.96
Parana	0.21b	133.4b	8.66b	0.91
Laboulaye	4.51c	107.5c	4.62c	0.94

[(1)]Means in column followed by same letter are not significantly different according to Fisher's protected LSD (0.05) test. [(2)]a_1: lower fresh weight asymptote; a_2: difference between upper and lower weight asymptote; a_3: rate associated with lower weight asymptote.

(Eleftherohorinos & Kotoula-Syka, 1995; Rosales Robles et al., 1999a, 1999b, 2001).

In the present study, reduced nicosulfuron rates provided an adequate Johnsongrass control, when applied at 4–5 leaf stage, but results varied according to the population considered. Populations from subhumid regions were significantly less susceptible to nicosulfuron from the 0.75X to 0.125X range. This effect was visualised in the values of regression coefficient of the non-linear models, in the time needed to obtain a 50% reduction of photosynthesis rate and stomatal conductance, and in the percentage of weed control. The time of 50% reduction of photosynthesis rate and stomatal conductance obtained in this experiment, for populations tested, was larger than that reported by Ferrel et al. (2003) for nicosulfuron applied at labeled rate. As nicosulfuron rates were reduced, a progressively higher time to reduce photosynthesis rate and stomatal conductance was needed for subhumid genotypes, as indicated by the significant population x herbicide rate interaction, which extended the period of competition by subhumid genotypes. This effect could be crucial to crop performance, considering that Johnsongrass populations reached the 4–5 leaf stage in the critical weed-free period of corn (V_4–V_7 stage) (Ghosheh et al., 1996).

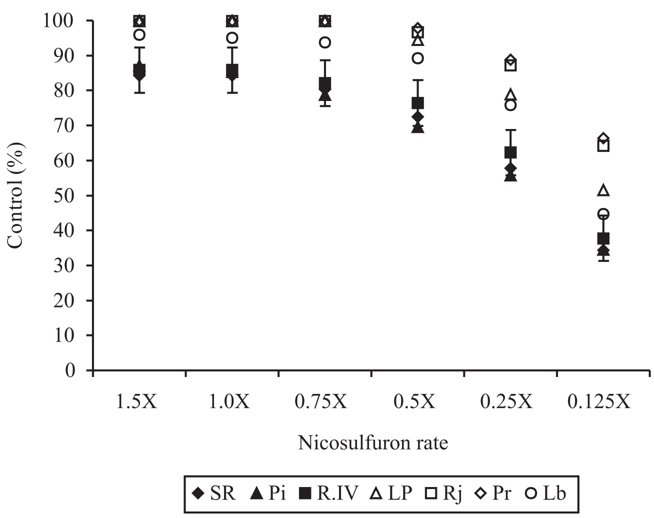

Figure 3. Control percentage of the seven Johnsongrass populations, in Santa Rosa (SR), General Pico (Pi), Río Cuarto (R.IV), La Plata (LP), Rojas (Rj), Paraná (Pr) and Laboulaye (Lb), compared with fresh weight of untreated plants. Vertical bars are LSDs ($p<0.05$).

In 16-week old untreated plants, significantly different values between genotypes from humid and subhumid populations were observed in fresh weight, total rhizome length and in the number of seed per plant (Figure 4 a, b and d). Number of nodes produced by rhizomes did not differ significantly between populations (Figure 4 c). The average fresh weight varied from 460.1 to 485.7 g in subhumid populations, while humid populations varied from 555.3 g to 594 g (Figure 4 a). Total average of rhizome length varied from 795 cm pl^{-1} to 897 cm pl^{-1} in subhumid populations, while in genotypes from humid regions it varied from 997.3 cm pl^{-1} to 1007.2 cm pl^{-1} (Figure 4 b). All four humid populations produced more seed per plant than the three subhumid ones (Figure 4 d).

No significant difference in fresh weight and seed number, between populations at 1.0X and 0.75X herbicide rates was observed (Figure 4 a and d), but significant difference ($p<0.05$) appeared between humid and subhumid populations, when 0.5X and 0.25X herbicide rates were considered. Laboulaye population showed an intermediate trend between these groups (Figure 4 a and d). Only the total rhizome length and the number of rhizome nodes, per plant, obtained at 0.25X herbicide rate, differ significantly ($p<0.05$) between population groups (Figure 4 b and c).

This study showed that three subhumid populations, with less sensibility to nicosulfuron rates, demonstrated substantial difference in fresh weight, total rhizome length and number of rhizomes nodes, in 16-week old plants (20 WAT). Previous studies had shown no Johnsongrass regrowth, after nicosulfuron application under different experimental conditions. Johnson et al. (2003) obtained no Johnsongrass regrowth after three WAT applying nicosulfuron at labeled rate, but the authors stated that regrowth would have occurred, if they had allowed the weed to remain longer in the experiment. Eleftherohorinos & Kotoula-Syka (1995) have indicated that no regrowth occurred within 8 to 12 WAT, when reduced nicosulfuron rates were used.

The important regrowth obtained in this experiment could be explained by leaf gas exchange registered during the evaluated period. The least leaf gas exchange of subhumid populations could result in a lower foliar absorption and translocation of nicosulfuron, than in humid populations, which would produce less control of subhumid populations, and would increase their ability to sprout and produce new aerial biomass. Moreover, exposing Johnsongrass subhumid populations to reduced

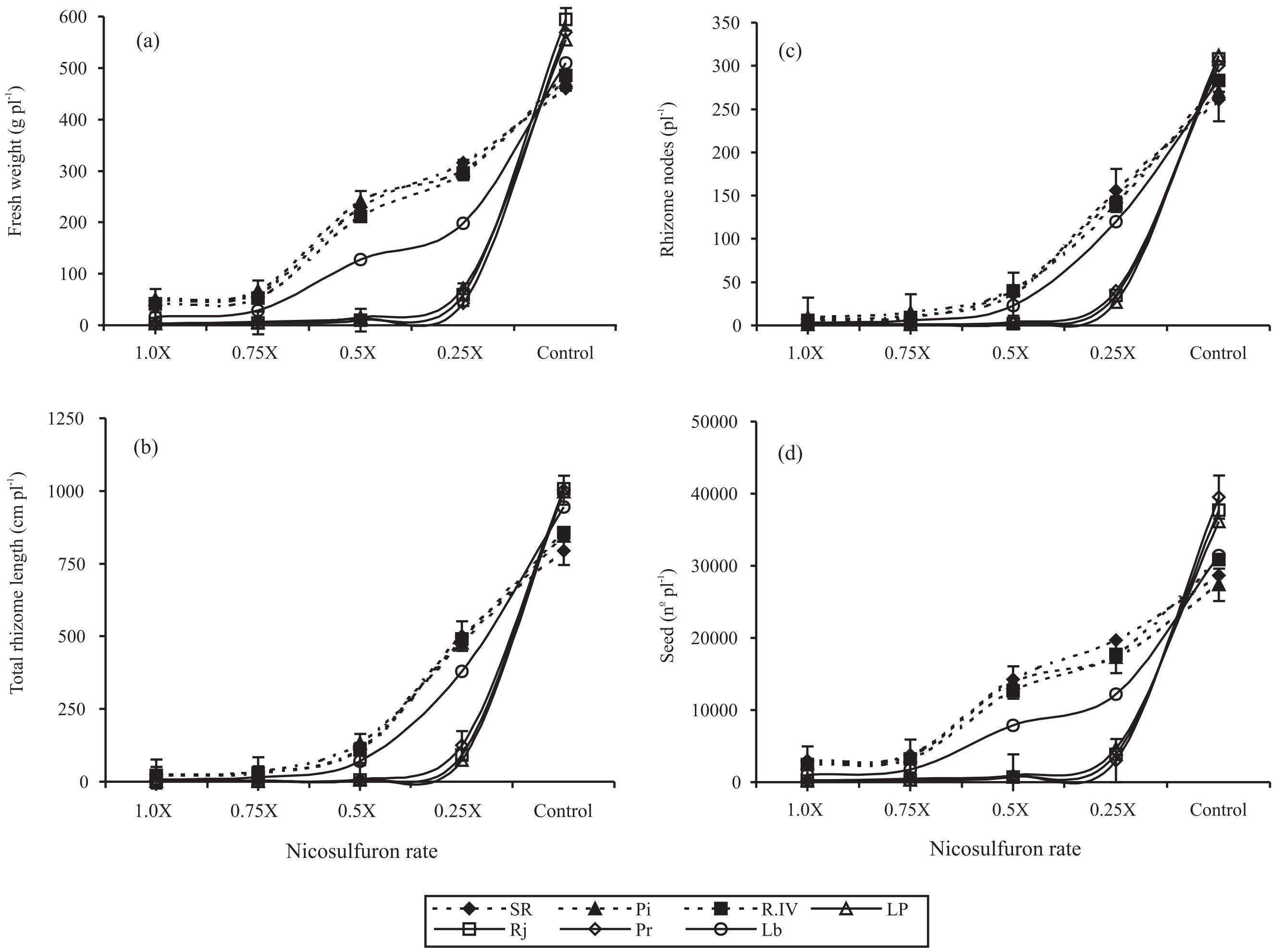

Figure 4. Fresh weight (a), total rhizome length (b), number of rhizome nodes (c), and number of seed (d) of seven Johnsongrass populations, in Santa Rosa (SR), General Pico (Pi), Río Cuarto (R.IV), La Plata (LP), Rojas (Rj), Paraná (Pr), and Laboulaye (Lb), after a growth period of 16 weeks (20 weeks after nicosulfuron application). Vertical bars are LSDs ($p<0.05$).

rates of nicosulfuron, mainly 0.5X and 0.25X, could result in both a progressively soil seed bank and rhizome population increase. Squire et al. (2000) found that both number of weed species and the total number of seeds increased with low herbicide rates.

The present study showed that the use of low rates of nicosulfuron is an effective management strategy to reduce Johnsongrass growth, and that the control efficiency was population related. The two classes of population analysed presented considerable variation in leaf gas exchange, susceptibility to nicosulfuron-reduced rates and regrowth after herbicide treatment. According to the registered results of Johnsongrass regrowth, the use of rates below one-half of nicosulfuron-labeled rate must be avoided in these environments. Moreover, an adequate knowledge of Johnsongrass population performance will be required to design an integrated weed management program using nicosulfuron at lower rates. Trends figured out in this work preclude the extrapolation of reduced rates use of herbicide from subhumid to humid region.

In order to minimize the important Johnsongrass regrowth, the long-term effects of this control strategy should be investigated.

Conclusions

1. The reduction in the fresh aboveground biomass of weed is higher in humid populations than in subhumid ones, in the fourth week after treatment, when reduced rates of nicosulfuron are applied.

2. The time needed to obtain a 50% reduction of photosynthesis rate and stomatal conductance is higher in subhumid than in humid populations.

3. The subhumid populations, with less sensibility to nicosulfuron rates, show substantial difference in fresh weight, total rhizome length and number of rhizome nodes.

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