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Tomo 54(2) - Diciembre 2022

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Production and physicochemical characterization of genotypes of *Eugenia uniflora* L.

Producción y caracterización fisicoquímica de genotipos de *Eugenia uniflora* L

Originales: *Recepción:* 29/10/2020 - *Aceptación:* 03/08/2022

Alex Danilo Monte Andrade [1], Elias Ariel Moura [2*],
Vander Mendonça [1], Luana Mendes Oliveira [1], Enoch Souza Ferreira [1],
Bruna Ester Ferreira Melo [1], Francisco Romário Andrade Figueiredo [1],
Marlenildo Ferreira Melo [1], Luciana Freitas Medeiros Mendonça [3]

Abstract

Pitanga (*Eugenia uniflora* L.) is an exotic fruit species of significant economic importance. However, due to genetic variability, its exploitation is hampered by the lack of homogeneous fruit production. In this scenario, this study aimed to select pitanga genotypes according to the physical and physicochemical parameters of fruits grown under semi-arid conditions. The study was developed at the Federal Rural University of the Semi-Arid Region with genotypes resulting from the open pollination of the pitanga variety 'Tropicana". Thirty-nine pitanga genotypes were evaluated for fruit mass, fruit length, fruit diameter, soluble solids (SS), titratable acidity (TA), ascorbic acid (AA), pH, and SS/TA ratio. The pitanga genotypes showed high variability. The clustering method separated the genotypes according to desirable traits. Genotype A12 showed the largest fruit sizes, whereas genotype A8 showed the highest SS and TA contents. Genotypes A2, A13, A34, and A39 showed fruits with the highest AT values. On the other hand, genotypes A11, A16, A45, A9, A26, and A44 showed the most significant contents of pH and SS/TA.

Keywords
agronomy in semi-arid conditions • genetic variability • Pitanga

1 Universidade Federal Rural do Semi-Árido. Department of Agronomic and Forest Sciences. Mossoró. RN. 59.625-900. Brazil.
2 Universidade Federal de Roraima. Department of Agriculture. Post-doctoral researcher. Capes. Boa Vista-RR. 69.300-000. Brazil. *eliasariel90@gmail.com
3 Universidade Federal de Campina Grande. Department of Agronomic and Forest Sciences. Patos-PB.58708-110. Brazil.

Resumen

Pitanga (*Eugenia uniflora* L.) es una fruta exótica de gran importancia económica por su alto potencial de uso. Sin embargo, debido a su variabilidad genética, su explotación se ve dificultada por no presentar producción de frutos homogénea. En este contexto, este estudio tuvo como objetivo seleccionar genotipos de pitanga de acuerdo con las características físicas y fisicoquímicas de los frutos en condiciones semiáridas. Se desarrolló en la Universidad Federal Rural del Semiárido y los genotipos se originaron a partir de la polinización abierta de la variedad pitanga 'Tropicana'. Se evaluaron 39 genotipos de pitanga con respecto a los siguientes rasgos: masa de fruto (g), longitud y diámetro de fruto (mm), sólidos solubles (SS, °Brix), acidez titulable (AT), ácido ascórbico (AA), pH y Relación SS/AT. Los genotipos de pitanga presentaron alta variabilidad. El agrupamiento permitió separar los genotipos de acuerdo con los rasgos deseables. El genotipo A12 presentó frutos de mayor calibre, y el genotipo A8 presentó los mayores contenidos de SS y AT. Los genotipos A2, A13, A34 y A39 presentaron altos valores de AT. Los genotipos A11, A16, A45, A9, A26 y A44 presentaron los contenidos más significativos de pH y relación SS / AT en los frutos.

Palabras clave

agronomia en condiciones semiáridas • variabilidad genética • Pitanga

Introduction

Brazil is one of the major worldwide centers of genetic diversity for fruit species. However, little is known about most of these species. In this scenario, pitanga (*Eugenia uniflora* L.) is a plant species of the family *Myrtaceae*, with an average shrub size (11) and good adaptation to semi-arid conditions (23).

There are several native and exotic fruit species with economic potential for cultivation. In this context, pitanga fruits stand out due to their exotic flavor and high vitamin contents, showing great potential for economic exploitation (16, 17). Most of these fruits are industrially processed or consumed fresh (4, 5, 29, 34).

Selection studies resulted in the release of the first Brazilian pitanga cultivar, 'Tropicana', with a mean annual yield of 20.8 kg ha^{-1} per year (for ten years) under dryland conditions. The mean fruit mass of this cultivar ranges from 3 to 4.5 g, with red-pulped fruits, soluble solids (SS) of 9 °Brix, titratable acidity (TA) of 2.2 g of citric acid per 100 g^{-1} fresh mass, and an SS/TA ratio of 4.1 (7, 8, 9).

However, despite the release of the 'Tropicana' variety, pitanga is still under domestication due to the existence of several genotypes in nature with the potential for agricultural use (6). These genotypes originated from asexually propagated plants and show high genetic variability, unevenness in their vegetative and reproductive cycles, and fruits with low physical and physicochemical quality.

Some preliminary studies have explored the genetic variability of plants aiming to select promising genotypes with desirable fruit traits (10, 13, 16, 23). However, scientific advances are still required for the genetic improvement of pitanga due to the high genetic variability of the species.

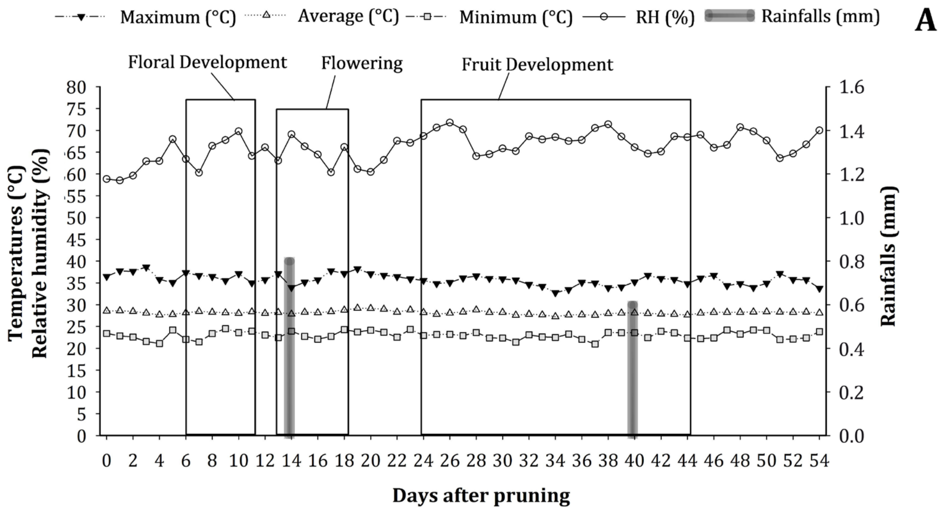
High variability in fruit species has become a significant problem for fruit quality, requiring the analysis of qualitative fruit traits by observing physical, chemical, and physicochemical properties to subsidize decision-making when selecting promising genotypes (30, 33). From this perspective, given the importance of analyzing fruit quality traits, the present study aimed to select pitanga genotypes according to the physical and physicochemical characteristics of fruits under semi-arid conditions.

Material and methods

Characterization of the area

Pitanga (*Eugenia uniflora* L.) genotypes were cultivated at the Rafael Fernandes Experimental Farm in the Federal Rural University of Semi-arid Region (UFERSA), Mossoró, Brazil (05°03'37'' S, 37°23'50'' W, 78 m a. s. l., flat relief). The regional climate, according to Köppen-Geiger, is classified as BSw'h', *i.e.*, tropical semiarid, with an average annual rainfall of 695.8 mm and an average annual temperature of 27.4°C, with two well-defined seasons: dry season (prolonged) and wet season (short and irregular). The natural vegetation is classified as Hyperxerophilic Caatinga (14).

The following meteorological data were recorded during the experimental period (2017 and 2018): rainfall; maximum, average and minimum temperature; and relative humidity (figure 1).



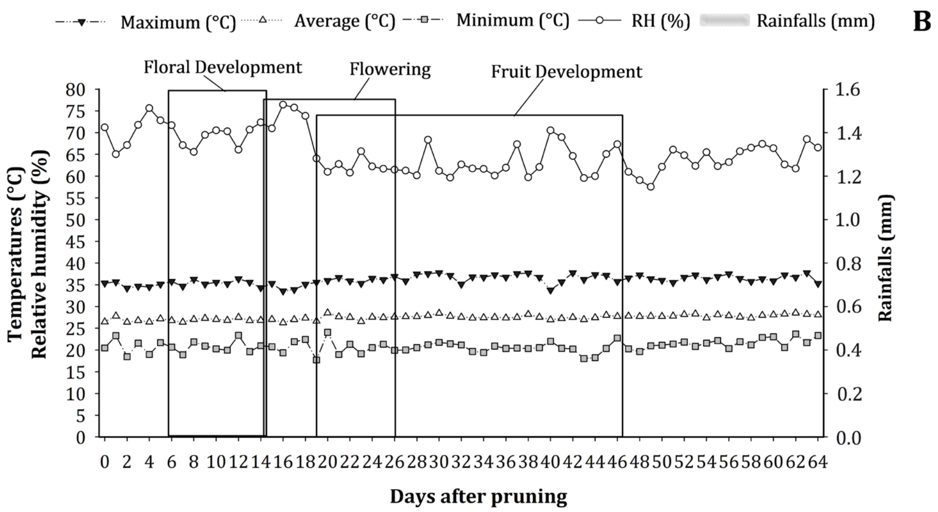


Figure 1. Maximum, minimum, and average temperature, rainfall, and relative humidity (RH) in 2017 (A) and 2018 (B) in Mossoró, Brazil.

Figura 1. Valores máximos, mínimos y promedio de temperatura, precipitación pluvial y humedad relativa (RH) en 2017 (A) y 2018 (B) en Mossoró, Brasil.

The climatic data were obtained from the ASA weather station (Meliponário Imperatriz; https://hobolink.com/p/004a83ec30555e14b039b2289d21d2f5) located at the Rafael Fernandes Experimental Station (experimental farm).

Plant material

In 2010, 39 pitanga genotypes were cultivated at a plant spacing of 3.0 x 1.5 m. The genotypes were obtained from the (figure 1, page 3), generation, without progeny test, and using open pollination for the 'Tropicana' cultivar obtained from the germplasm bank of the State University of Northern Rio de Janeiro (UENF), Campos dos Goytacazes, Rio de Janeiro, Brazil.

Crop management was performed according to Lira Júnior *et al.* (2007). Before pruning, plants were subjected to water stress in an irrigation suspension for 33 days to induce the reproductive stage. Pruning was first carried out on June 10, 2017, in the first cycle, and for the second time on July 27, 2018.

Fruit quality

The fruits were harvested weekly and evaluated for physical and physicochemical traits.

The parameters of fruit length, diameter, and mass were determined by evaluating 20 fruits per plant. All fruits were harvested at the same maturation stage and measured with a digital caliper (± 0.01 mm). Fruit mass was determined in an analytical balance, and the results were expressed as grams (± 0.01 g).

Twenty fully ripe fruits were evaluated per plant to determine the chemical attributes. The soluble solids (SS) were determined directly in the homogenized pulp juice using a digital refractometer (model PR - 100, Palette, Atago Co, LTD., Japan), with the results expressed as °Brix (2).

Titratable acidity (TA) was determined by titration using 1 g of pulp transferred to a 125 mL Erlenmeyer flask containing 49 mL water. Afterward, titration was performed with a previously standardized 0.1 M NaOH solution until reaching pH 8.1, with the results expressed as g of citric acid per 100 g^{-1} pulp (2). The SS/TA ratio was determined by the ratio of soluble solids to titratable acidity.

The hydrogen potential (pH) was determined using a digital potentiometer with automatic temperature adjustment (Model mPA-210 Tecnal®, Brazil), previously calibrated with buffer solutions at pH 7.0 and 4.0 (2).

Vitamin C (AA) was determined by titration with Tilman's solution (2,6 dichlorophenol indophenol at 0.02%) by diluting 1 g of sample in 50-mL volumetric flasks containing 0.5% oxalic acid (31). The results were expressed as mg of ascorbic acid per 100 g^{-1} pulp.

Statistical analysis

The data on the agronomic traits of each genotype were analyzed by descriptive statistics. Multivariate data analysis was performed using Principal Component Analysis (PCA). The genetic distances were calculated by considering the eight traits. The genotypes were grouped by Ward's hierarchical clustering method based on the genetic distance matrices (Ward. D) (18, 19). The analyses were performed with the R software (26).

Results

Most genotypes evaluated showed moderate to high variability, with low variability occurring only in fruit pH (table 1, page 5 and table 2, page 6).

The fruit mass (FM) ranged from 1.4 g to 4.7 g, with a mean of 2.52 g for the first year (2017). FM showed significant variations in the second year (2018) ranging from 1.33 to 6.1 g, with a mean of 2.59 g. The variation amplitude for fruit length (FL) ranged from 9.8 to 16.90 mm (2017) and from 11.11 to 17.61 mm (2018), with the respective means of 12.73 mm and 13.35 mm for the two years. Fruit diameter (FD) ranged from 13.40 mm to 23.20 mm (2017) and from 13.98 mm to 24.00 mm (2018), with mean values of 17.22 mm and 17.55 mm, respectively (table 1, page 5).

Table 1. Physical parameters of the fruits of pitanga (*Eugenia uniflora* L.) genotypes harvested at the predominantly red stage.

Tabla 1. Características físicas de frutos de genotipos de pitanga (*Eugenia uniflora* L.) recolectados en la etapa predominantemente roja.

Genotypes	FM[1] (g)		FL[2] (mm)		FD[3] (mm)	
	2017	2018	2017	2018	2017	2018
A1	2.4	2.3	12	12.49	16.8	16.02
A2	3.8	2.21	14.2	12.48	19.8	17.24
A3	2.1	2.2	11.4	13.28	16.1	17.43
A4	2.9	2.11	12.9	12.63	17.8	17.13
A6	1.8	2.55	10.5	12.85	14.8	17.63
A8	-	4.2	-	14.48	-	21
A9	2.4	2.8	10.8	12.61	16.2	17.27
A10	1.8	3.05	10.8	13.74	15.4	17.58
A11	1.9	2.4	15	12.86	22.5	15.1
A12	4.7	6.1	15	17.61	22.2	24
A13	2.4	3.45	16.9	14.42	23.2	19.85
A14	1.4	2.1	9.8	13.26	13.4	16.61
A15	2.1	2.15	11.9	14.51	15.7	17.65
A16	-	1.85	-	11.21	-	14.61
A17	1.7	2.55	16.7	13.78	14.4	19.92
A18	4.2	2.75	14	12.05	20.4	17.63
A19	2.1	1.75	11.3	11.86	15.9	17.29
A20	-	3.11	-	13.61	-	18.23
A21	2.3	2.35	11.4	12.96	16.6	17.02
A23	2.95	3	14.7	14.26	19.3	19.07
A24	2.8	3.3	13.3	14.47	18.3	19.27
A25	2.8	3.05	11.5	12.83	16.7	1843
A26	1.8	1.8	11.4	12.98	14.8	15.41
A27	2.2	2.3	11.3	11.77	16	16.42
A28	2.1	2.65	11.6	13.08	15.2	16.58
A29	-	2.35	-	13.1	-	16.53
A31	2.4	2.5	12.7	13.57	16	17.67
A32	3.2	2.2	14.8	14.53	19	15.68
A33	-	1.33	-	11.11	-	13.98
A34	3	2.9	15.9	16.21	16.9	18.26
A39	2.6	2.7	12.9	15.53	17.2	19.41
A40	-	2.35		13.39		16.64
A41	2.9	2.4	13.1	12.77	18.1	17.65
A42	2.2	2	11.7	13.71	15.6	16.07
A43	2.8	3.8	11.9	14.28	16.9	20.14
A44	-	1.95	-	12.58	-	16.35
A45	-	1.8	-	11.84	-	14.2
A46	1.8	2.4	10.6	12.4	15.4	16.95
A47	-	2.2	-	15.32	-	18.6
Median[4]	2.52	2.59	12.73	13.35	17.22	17.55
CV%[5]	29.52	31.27	15.06	10.18	14.18	11.28
SD[6]	0.74	0.81	1.92	1.36	2.44	1.98

[1] Fruit mass (g); [2] Fruit length (mm); [3] Fruit diameter (mm); [4] Median; [5] Coefficient of variation; [6] Standard deviation.

[1] Masa de fruta (g); [2] Longitud del fruto (mm); [3] Diámetro del fruto (mm); [4] Media; [5] Coeficiente de variación; [6] Desviación Estándar.

Table 2. Chemical parameters of the fruits of pitanga (*Eugenia uniflora* L.) genotypes harvested at the predominantly red stage.

Tabla 2. Características químicas de frutos de genotipos de pitanga (*Eugenia uniflora* L.) recolectados en la etapa predominantemente roja.

Genotypes	SS [1]		TA [2]		AA [3]		pH [4]		SS/TA [5]	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
A1	13.3	9.7	1.13	1.2	43.43	64.96	3.56	3.77	11.7	8.11
A2	13.4	9.2	2.29	1.72	52.36	34.3	3.26	3.61	5.8	5.35
A3	13	9.4	1.37	1.37	54.43	49.94	3.62	3.68	9.5	6.86
A4	11.1	7.85	0.83	1.25	44.62	49.94	3.41	3.47	13.3	6.28
A6	11.6	11.25	1.07	1.3	60.45	52.39	3.49	3.55	10.8	8.65
A8	-	15.2	-	0.76	-	214.16	-	4.14	-	20.12
A9	17.8	13.1	1.05	1.07	52.5	101.7	3.99	3.76	17	12.2
A10	10.5	7.6	0.8	1.07	69.9	65.49	3.72	3.53	13.2	7.12
A11	13.2	11.2	0.66	0.68	53.69	65.39	4.05	3.88	20.1	16.37
A12	11.4	9.65	2.23	1.42	43.49	97.88	3.22	3.24	5.1	6.78
A13	11.3	10.5	2.45	1.61	44.19	49.04	3.19	3.7	4.6	6.53
A14	8	8.85	0.71	0.74	63.18	91.53	3.64	4.14	11.2	11.89
A15	10.8	11.15	1.48	1.31	61.88	106.19	3.6	3.82	7.3	8.54
A16	-	10.45	-	0.91	-	70.17	-	3.89	-	11.43
A17	11.7	7.8	0.62	0.52	61.22	50.36	3.83	3.99	18.9	14.94
A18	15	10	0.94	0.82	43.93	57.11	3.71	3.78	15.9	12.13
A19	11.2	7.3	1.19	0.95	72.35	49.16	3.59	3.72	9.4	7.67
A20	-	7.2	-	0.59	-	69.14	-	3.84	-	12.12
A21	13.1	10.85	1.07	1	62.35	61.52	3.59	3.83	12.2	10.86
A23	11.6	10.45	0.93	1.07	68.7	87.23	3.61	3.73	12.4	9.73
A24	12.2	9.05	0.84	1.25	71.48	32.55	3.81	3.61	14.5	7.22
A25	15.1	10.7	1.09	0.8	51.09	60.58	3.51	4.08	13.8	13.33
A26	17.6	12.4	1.64	0.87	62.88	69.63	3.66	3.9	10.7	14.2
A27	11.7	12.4	0.68	1.24	68.5	49.4	3.95	3.82	17.3	9.99
A28	11.1	10.6	0.73	0.71	44.23	65.56	3.85	3.94	15	14.87
A29	-	10.4	-	0.9	-	34.63	-	3.82	-	11.54
A31	13.1	8.05	1.23	0.63	61.2	34.36	3.48	4.18	10.6	12.69
A32	12	8.15	1.75	0.76	62.75	33.23	3.29	4.05	6.9	10.7
A33	-	9.65	-	1.19	-	61.2	-	3.46	-	8.12
A34	10.2	11.15	1.87	1.33	53.62	60	3.29	3.45	5.5	8.37
A39	13.8	9.2	1.61	1.63	52.75	50.56	3.45	3.49	8.6	5.66
A40	-	6.95	-	0.79	-	68.62	-	3.64	-	8.81
A41	12.4	10.45	0.84	1.19	52.09	86.65	3.95	3.87	14.7	8.81
A42	10.7	8.7	1.47	1.13	51.85	34.69	3.46	3.68	7.3	7.7
A43	11.1	10	0.99	1.29	80.02	92.44	3.77	3.59	11.2	7.76
A44	-	13.2	-	1	-	60.71	-	3.86	-	13.26
A45	-	9.2	-	0.62	-	51.54	-	3.89	-	14.76
A46	10.1	10.6	1.24	0.97	63.11	106.05	3.5	3.76	8.1	10.92
A47	-	12.45	-	1.13	-	67.39	-	3.72	-	11.05
Median	12.3	10.05	1.22	1.05	57.6	66.86	3.6	3.77	11.42	10.34
CV%	16.9	18.1	40.78	28.57	17.16	47.5	6.44	5.57	36.36	32.01
SD	2.07	1.82	0.5	0.3	9.88	31.76	0.23	0.21	4.15	3.31

[1] Soluble Solids (°Brix); [2] Titratable Acidity (g citric acid 100 g^{-1}); [3] Ascorbic Acid (mg of ascorbic acid per 100 g^{-1} pulp); [4] pH; [5] SS/TA.

[1] Sólidos solubles (°Brix); [2] Acidez titulable (g de ácido cítrico 100 g^{-1}); [3] Ácido ascórbico (mg de ácido ascórbico por 100 g^{-1} pulpa); [4] pH; [5] SS / TA.

The higher the diameter of Pitanga fruits, the higher the fruit mass. The pitanga genotypes showed positive correlations between FM and FD ($r= 0.66$, $p<0.0001$) in 2017 and ($r= 0.87$, $p>0.0001$) in 2018.

The solids soluble (SS) ranged from 8.0 to 17.8 °Brix (2017) and from 7.2 to 15.20 °Brix (2018), with means of 12.3 °Brix and 10.05°Brix, respectively. For Titratable Acidity (TA), the values ranged from 0.62 to 2.45 g of citric acid per $100g^{-1}$ (2017) and from 0.52 to 1.7 g of citric acid per $100g^{-1}$ (2018), with mean values of 1.23 and 1.5 g of citric acid per $100g^{-1}$, respectively. The SS/TA ratio ranged from 4.6 to 20.10 (2017) and from 5.35 to 20.12 (2018), with mean values of 11.42 and 10.34 for the two cycles (table 2).

The AA means in the two seasons were 57.61 and 66.86 mg of ascorbic acid per 100 g^{-1} pulp. The variations among genotypes ranged from 43.43 to 80.02 (2017) and from 32.55 to 214.16 (2018). The variations were minor for fruit pH, with mean values of 3.6 (2017) and 3.77 (2018) (table 2, page 6).

The principal component analysis explained 50.19% of data variability, with PC 1 and PC 2 explaining 33.31% and 16.88% of data variability, respectively (figure 2).

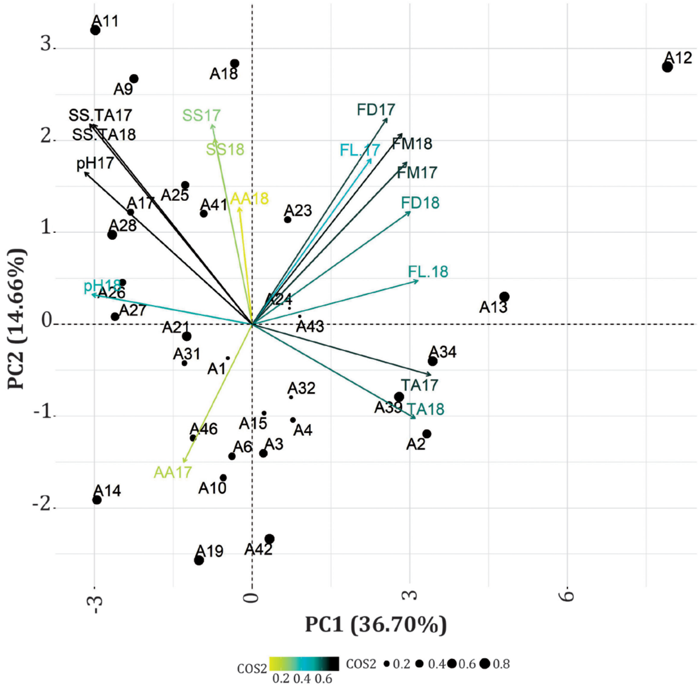


Figure 2. Principal component analysis (PCA) performed for pitanga (*Eugenia uniflora* L.) genotypes with regard to physical and physicochemical fruit parameters.

Figura 2. Análisis de componentes principales (ACP) de las características físico-químicas de frutos de genotipos de pitanga (*Eugenia uniflora* L.).

The variables that showed the highest correlations with PC 1 were TA ($r=0.79$; $p<0.001$), TA18 ($r=0.68$; $p<0.001$), FM ($r=0.67$; $p<0.001$), FL18 ($r=0.67$; $p<0.001$), DT17 ($r=0.60$; $p<0.005$), and DT18 ($r=0.60$; $p<0.005$), whereas the variables with negative correlations were pH17 ($r=-0.74$; $p<0.0001$), SS/TA17 ($r=-0.71$; $p<0.0001$), pH18 ($r=-0.64$; $p<0.0005$), and TA18 ($r=-0.60$; $p<0.0005$). For PC2, the variables that contributed most were AA ($r=-0.69$; $p<0.0001$), FM ($r=-0.65$; $p<0.0005$), and SS/TA ($r=-0.64$; $p<0.0001$). Genotypes A12, A13, A2, and A34 contributed most to PC1. For PC2, genotypes A8, A19, A33, and A42 showed the highest contributions.

Genotype 12 showed larger fruits, whereas genotype A8 showed fruits with superior organoleptic qualities, with high SS, AA, and SS/TA contents (figure 3, page 8).

These genotypes were individually isolated and formed groups 1 and 3 in the clustering analysis. Genotypes A2, A13, A34, and A39 showed the highest TA values and were similar to each other, belonging to group 2. However, these genotypes were negatively correlated with the pH, SS/TA, and AA contents, whose higher values were present in the genotypes of group 8 (figure 3, page 8; table 3, page 8).

Genotypes A43, A23, and A24 formed group 4, with intermediate values for fruit size. The genotypes that formed group 5 showed intermediate values for TA and AA. In general, the second year increased the physical and physicochemical characteristics of pitanga fruits (figure 2). The genotypes of group 6 showed similar values for all traits evaluated, whereas group 7 showed intermediate values for pH, SS/TA, and AA, in addition to the lowest TA values (figure 3, page 8; table 3, page 8).

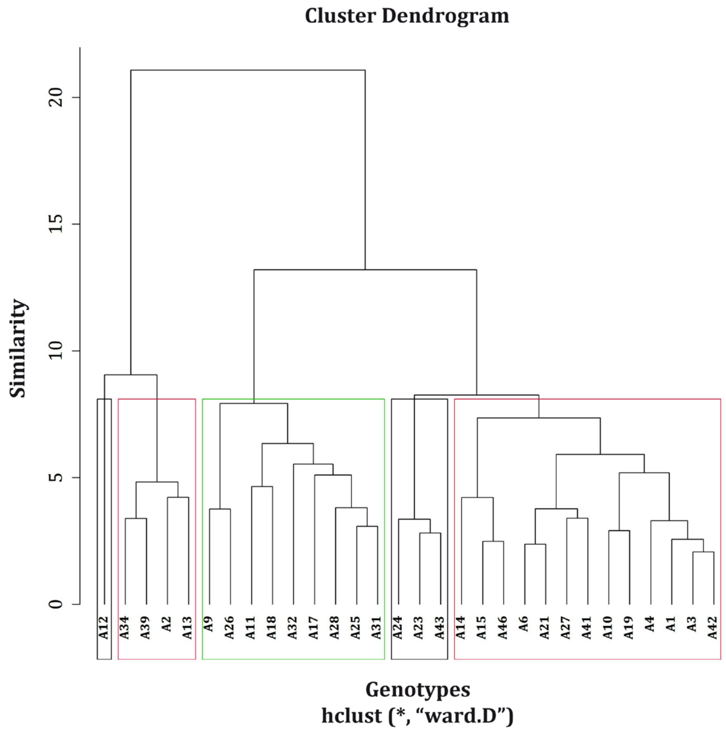


Figure 3. Cluster analysis by the Ward. D grouping method and using the Euclidean distance to arrange the 39 pitanga (*Eugenia uniflora* L.) genotypes into eight groups.

Figura 3. Análisis de Cluster por el método de agrupación de Ward utilizando la distancia Euclidiana de 39 genotipos de pitanga (*Eugenia uniflora* L.).

Table 3. Clustering of pitanga (*Eugenia uniflora* L.) genotypes based on the genetic similarity matrix calculated using eight agronomic traits.

Tabla 3. Agrupación de genotipos de pitanga (*Eugenia uniflora* L.) a partir de la matriz de similitud genética calculada a partir de 8 características agronómicas.

Group	Genotype
1	A12
2	A34, A39, A2, A13,
3	A9, A26, A11, A18, A32, A17, A28, A25, A31
4	A24, A23, A43
5	A14, A15, A46, A6, A21, A27, A41, A10, A19, A4, A1, A3, A42

Discussion

The multivariate analysis allowed the selection of the best genotypes according to physical and physicochemical parameters. Fresh fruit commercialization is usually based on physical characteristics, with larger fruits being more attractive to customers (13). All fruits of the pitanga genotypes evaluated were larger in diameter than in length, confirming previous reports (10, 28).

Variations similar to the present study were observed by Dias *et al.* (2011) when characterizing pitanga genotypes, which, in turn, were higher than the variations observed by Avila *et al.* (2009) and Castro *et al.* (2020). These variations are mainly influenced by environmental conditions and the behavior of each genotype in the environment (23).

The largest pitanga fruits ever recorded were observed by Bezerra *et al.* (2004), with a mean of 4.5 g, and by Fonseca *et al.* (2009), with 4.24 g in genotypes grown under mild climatic conditions. Fruits with different sizes as a function of climatic conditions were observed by Castro *et al.* (2020), who noticed that pitanga fruits grown in Argentina are much smaller than those collected in Brazil.

The SS values of the pitanga genotypes showed satisfactory values, similar to those observed by Bezerra *et al.* (2004) and by Dias *et al.* (2011), highlighting the role of this species as an excellent option for the industry (5, 9, 29, 34). Fruits with high SS contents are usually preferred for both fresh consumption and industrialization since they provide higher yields after processing, given the higher nectar content produced per pulp volume (32). The general means obtained for SS were similar to the mean contents observed by Bezerra *et al.* (2004) and higher than those obtained by Dias *et al.* (2011) and Batista *et al.* (2014). This variation in the SS contents is probably due to genetic and environmental factors.

For TA, the wide variation observed may be related to the genetic components, which, along with the environmental factor, expressed the phenotype differently for this trait (6). Genotypes with TA contents above 1.00% are preferred by the agroindustry since there is no need to add acid to preserve the pulp and prevent microorganism development (20, 24).

The chemical composition of pitanga fruits can be affected by factors such as their origin, climatic conditions, the genotypes analyzed, and the harvest season (10, 17, 23). The vitamin C contents were above those observed by Batista *et al.* (2014) and Sanches *et al.* (2017). The ascorbic acid content naturally present in fruits serves as a nutritional parameter due to the high antioxidant power of this component in preventing and combating several diseases, with a recommended daily allowance (RDA) of 75 mg day^{-1} for adult women and 90 mg day^{-1} for adult men (1).

Fruit pH showed ideal values for both fresh consumption and the industry. Low pH values favor fruit preservation, avoiding the addition of acids and preventing microorganism development (3). Therefore, high pH values are essential for fresh consumption in order to provide fruits with lower acidity. Similar pH values were observed by Bezerra *et al.* (2004) and Batista *et al.* (2014).

The SS/TA ratio is one of the best tools to evaluate fruit flavor, being more representative than the individual analysis of soluble solids and acidity (11). In this study, the ST/TA ratio showed significant variation among genotypes (coefficient of variation of 36.36 and 32.01%), with general means of 11.42 and 10.34 for the two cycles studied. These values agree with those observed in other studies (6, 9, 13).

Furthermore, the SS/TA ratio is a better parameter to assess the maturation stage than the isolated measurement of sugars and acidity, in addition to being an important parameter to evaluate fruit quality and one of the most usual ways to evaluate the flavor of food products (21). Therefore, fruits with high soluble solids contents and low acidity are preferable for a high SS/TA ratio.

The high genetic variability observed in 2017 and 2018 demonstrated that the preliminary results were satisfactory, especially compared to the preliminary results obtained by Bezerra *et al.* (1997). The occurrence of variation in the accessions is very important for plant breeders since this is their raw material (25).

Fruit traits should be analyzed for four or five consecutive production cycles according to the genetic improvement program of fruit species developed by Embrapa Clima Temperado (12) to select superior genotypes. This number of evaluations is considered adequate and effectively predicts the actual value of the individuals, showing more than 80% reliability for all traits and indicating that the traits can be used in the phenotypic selection of superior pitanga genotypes.

Conclusion

The pitanga genotype A12 showed larger fruit sizes, whereas genotype A8 showed the highest SS and AA contents in the second year.

Genotypes A2, A13, A34, and A39 showed the highest TA contents in pitanga fruits. The genotypes of group 8 (A11, A16, A45, A9, A26, and A44) showed the most significant pH and SS/TA ratio values in the pitanga fruits.

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Line x tester analysis to estimate combining ability in grain sorghum (*Sorghum bicolor* L.)

Análisis línea x probador para estimar la aptitud combinatoria en sorgo de grano (*Sorghum bicolor* L.)

Héctor Williams-Alanís [1], Ulises Aranda Lara [2*], Gerardo Árcos Cavazos [1], Francisco Zavala Garcia [3], Marisol Galicia Júarez [4], María del Carmen Rodríguez Vázquez [3], Jorge Elizondo Barrón [1]

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Abstract

Sorghum in Mexico ranks third in grain production. This study aimed to estimate general combining ability (GCA) and specific combining ability (SCA) for commercial and experimental sorghum grain parents and hybrids. The combining ability was estimated using the line x tester method described by Kempthorne. The experiment was established during the spring-summer 2018 cycle at INIFAP, Las Huastecas, México. It consisted of five lines, eight testers and 40 hybrids; in a randomized block design with three replications. The ANOVA showed highly significant differences for lines, testers, and line x testers, suggesting the existence of a broad base of genetic variability. GCA and SCA differences were statistically significant for grain yield, specific grain weight and plant height, indicating additive gene relevance, dominance and epistasis. For grain yield, the experimental lines: RB214A, RB225A and RB248A, and the testers RB133 and RB221, resulted significantly higher in GCA and superior to commercial RB225A line and RTx430 and RTx437 testers. Seventeen experimental hybrids were found to have significantly higher in SCA and were superior to INIFAP commercial hybrids.

Keywords
Sorghum bicolor L. • plant breeding • hybrids • parental lines • grain production

1 Ex-Investigador del Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP). Campo Experimental Rio Bravo. Programa de Sorgo.
2 Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, (INIFAP). Campo Experimental Rio Bravo. Programa de Sorgo.* aranda.ulises@inifap.gob.mx
3 Universidad Autónoma de Nuevo León (UANL). Facultad de Agronomía.
4 Universidad Autónoma de Baja California. Instituto de Ciencias Agrícolas. Carretera a Delta/ Oaxaca s/n. Ejido Nuevo León. Valle de Mexicali. Baja California. CP 2175.

Resumen

El sorgo en México ocupa el tercer lugar en producción de grano. El objetivo del estudio fue estimar la aptitud combinatoria general (ACG) y especifica (ACE) en progenitores e híbridos comerciales y experimentales de sorgo para grano. La aptitud combinatoria se estimó mediante el método de línea x probador descrito por Kempthorne. El experimento se estableció durante el ciclo primavera-verano 2018 en INIFAP, Huastecas, México. Consistió de cinco líneas, ocho probadores y 40 híbridos; en un diseño de bloques al azar con tres repeticiones. El ANOVA detectó diferencias altamente significativas para líneas, probadoras y línea x probador; sugiriendo la existencia de una amplia base de variabilidad genética. Para rendimiento de grano, peso específico de grano y altura de planta, la ACG y ACE, fueron significativas lo cual indica la relevancia de genes aditivos de dominancia y epistasis. Para rendimiento de grano las líneas experimentales: RB214A, RB225A y RB248A y, los probadores RB133 y RB221, fueron altamente significativos en ACG y fueron superiores a la línea RBSBA25 y los probadores RTx430 y RBTx437 comerciales. Se encontró diecisiete híbridos experimentales con diferencias significativas en ACE y fueron superiores que los híbridos comerciales del INIFAP.

Palabras clave

Sorghum bicolor L. • mejoramiento genético • híbridos • progenitores • producción de grano

Introduction

In México, sorghum [*Sorghum bicolor* (L.) Moench] is the third largest grain crop produced after corn, *Zea mays* (L.) and common bean, *Phaseolus vulgaris* (L.) (21). Tamaulipas is the main sorghum-producing state, contributing in 2017 with 2,205,000 tons of grain, equivalent to 45.45% of national production (1). However, this supply is not enough for the national demand, making it necessary to increase productivity (18).

The discovery of cytoplasmic male sterility by Stephens and Holland (1954), was of vital importance for the commercial production of hybrid seed, allowing significant production improvement (19). In the United States of America, 35% to 40% of total profit obtained in grain production, is attributed to this technology (4). In this sense, a successful hybridization program largely depends, on selecting the proper parental lines and knowing the different types of gene action (9). General combining ability (GCA) and specific combining ability (SCA) are key tools in plant genetic improvement (3, 15). The line x tester mating method for GCA and SCA determination suggested by Kempthorne (1957) is appropriate for parent and higher hybrid identification (7). In this context, using productive hybrid seed with enhanced environmental adaptation has been fundamental for obtaining higher yields. Available Mexican seed varieties provide job positions in production activities while reducing capital flight (26). In this sense, The Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP), Río Bravo Experimental Station, Tamaulipas, México, began working on genetic sorghum breeding in 1973, generating varieties and hybrids for northern México (28): RB-2000, RB-2020, RB-3030, RB-3006, RB-4000, RB-Patrón, RB-4040, RB-Huasteco, RB-Norteño; and RB-Paloma, RB-Gaviota, RB-Williams and Arcos varieties.

The objective of this study was to estimate general combining ability (GCA) effects for females and males, and specific combining ability (SCA) effects for grain sorghum hybrids, identifying high-yield hybrids with earliness and adequate harvest height.

Materials and methods

The Genetic material considered in this study comprised the commercial lines: A/B: SBA12/SBB12, parents of RB-3030; SBA25/SBB25 parents of RB-4000, RB-Patron and RB-Huasteco hybrids. R Lines (testers): RTx430 (11) and RTx437 (17), originated at Texas

A&M University. INIFAP experimental lines A/B: RB214A/RB214B, RB225A/ RB225B, RB248A/RB248B, and testers: RB128, RB133, RB135, RB221, RB256 and RB373. These genotypes were generated by hybridization in 2003. Maintainer lines were generated from SBB-25, parent of RB-Patrón hybrid (27), LRB-118B, parent of RB-4040 hybrid (25) and VAR-B, generated by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India. Experimental testers were generated from crosses between RTx437, LRB-210 and SBR-24. RTx437, originated in Texas A&M University (16); LRB-210, parent of RB-4040, INIFAP hybrid (24); SBR-24, parent of RB-4000 INIFAP hybrid. In F2 generations, row by panicle selection or pedigree was performed along five generations for large panicle length, higher grain volume, plant height, non-senescent plant, charcoal rot tolerance [*Macrophomina phaseolina* (Tassi) Goid], Head smut [*Sporisorium reilianu* (Kühn)] Langdon and Fullerton, and foliar diseases. In Marin Experimental Station, Marin, Nuevo León, México, Universidad Autónoma de Nuevo León (UANL), from 2010 to 2017, the described selection process continued while sterilization of best maintainer lines (B) was carried out, by 7 generations backcrosses.

The genetic material was increased at the mentioned Experimental Station, during spring-summer 2017 and fall-winter 2017-2018. A and B lines and R were increased, and F1 hybrids were developed under line × tester (5 × 8) crossing scheme. The experiment was established under rain-fed conditions during the spring-summer 2018 in INIFAP, Las Huastecas Experimental Station, located at Altamira municipality, Tamaulipas, Mexico; 22°33’ LN/ 98°09’ LW, and 20 m above sea level. It has a warm sub-humid climate (Aw0) with summer and winter rain (5); average annual temperature of 24.5°C and 842 mm annual rainfall (6). This experiment consisted of 40 possible hybrid combinations, including INIFAP´s commercial hybrids; RB-3030, RB-Patrón and RB-Huasteco, five fertility maintaining lines, eight testers and the commercial hybrids Pioneer P83P27 and P85P20. The B lines or fertility-maintaining lines were used to determine maternal *per se* performance.

The experimental design was a randomized block with 55 treatments and three replications, distributed in a one-row plot of 5 m long and 0.80 m apart. Sowing was on August 15, 2018. Fertilization was 90-40-00 with triple superphosphate and ammonium nitrate. Thinning was done 20 days after emergence leaving a population of 250 thousand plants ha^{-1}. Weed control was carried out at 10, 20 and 25 days after plant emergence. The yellow aphid [*Melanaphis sacchari* (Zehntner)] was controlled with Toreto (Sulfoxaflor) 21.8% at a dose of 0.06 l ha^{-1}.

Days to flowering (DF) were observed at 50% flowering. One week before physiological maturity, the following traits were measured: plant height (PH, in cm, from the ground to the panicle apex), panicle length (PL, in cm, from the base to the apex), and exertion length (EL, in cm, from the ligule of the flag leaf to base panicle). The entire plot was harvested and threshed. Then, grain moisture was determined, estimating grain yield (GY). Results were reported in t ha^{-1} (GY, at 12% moisture) and specific grain weight (SGW, in kg per hectoliter). Line × Tester analysis was done according to Kempthorne (8) estimating general combining ability (GCA) effects for females and males and specific combining ability (SCA) effects for hybrids. Statistics were carried out with R platform (16).

Results and discussion

The ANOVA (table 1, page 15), revealed highly significant differences ($p \leq 0.01$) between parents and hybrids for all traits. In this regard, Mohammed (2009) found similar results in forage sorghum for days to flowering and plant height. In parents x hybrids, highly significant differences were observed in DF, PH, PL and GY variables, while significant differences resulted for EL. In lines x tester, highly significant differences were observed in DF and PH, while significant differences were found in GY. Likewise, highly significant differences in EL and PL were found for treatments, parents, crosses and lines. Results indicate a broad base of genetic variability among the used germplasm, favoring an appropriate selection of parents and hybrids.

SGW: specific grain weight (kghl^{-1}); DF: days to flowering; PH: plant height (cm); EL: exertion length (cm); PL: panicle length (cm); and GY: grain yield (kgha^{-1}). C.V.; coefficient of variation (%). *, **: Significant at 0.05 and 0.01% probability levels, respectively.

SGW: peso específico del grano (kg hl^{-1}); DF: días a floración; PH: altura de planta (cm); EL: longitud de excersión (cm); PL: longitud de panícula (cm); y GY: rendimiento de grano (kg ha^{-1}). C.V.: coeficiente de variación (%). *, **: Significativo al nivel de 0,05 y 0,01% de probabilidad, respectivamente.

Table 1. Line x tester ANOVA for agronomic traits. INIFAP, Las Huastecas Experimental Station, Altamira, Municipality, Tamaulipas, México. 2018, Spring-Summer cycle.

Tabla 1. Análisis de varianza por el método de línea x probador de las variables agronómicas. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

Source	D. F.	SGW (kg/hl^{-1})	DF (days)	PH (cm)	EL (cm)	PL (cm)	GY (kg/ha^{-1})
Replications	2	2832.31	32.96*	345.82*	37.09*	398.35**	5446408**
Treatments	53	5794.01**	32.45**	1605.31**	22.41**	32.42**	5837857**
Parents	13	11821.49 **	31.23**	1246.35**	28.15**	37.75**	5279849**
Parents x crosses	1	51.82	205.91**	25055.93**	47.78*	704.26 **	74015669**
Crosses	39	3932.08**	28.41**	1123.67**	19.85**	13.42**	4275711**
Lines	4	15762.24**	130.86**	1155.88**	58.68**	42.90**	5378067**
Testers	7	6652.71**	63.54**	4298.37**	39.26**	26.71**	14027875**
Lines x testers	28	1561.90	4.99**	325.39**	9.45	5.89	1680190*
Error	109	1656.18	9.44	98.58	9.99	8.45	1068553
C.V.		5.9	5.07	5.7	26.61	11.6	22.8

General combining ability (GCA) in lines (table 2), showed four out of five lines with significant values (p≤0.01) for GY, of which three corresponded to experimental lines. For SGW, PH and PL, three out of five lines showed high GCA values. For GY, genotypes RB214A, RB225A, RB248A and SBA-25 showed highly significant values (p≤0.01). In addition, the experimental line RB225A presented highly significant values for SGW, DF, PH and EL. For PH, negative GCA values are desirable (9), given that low-PH sorghums are preferred. The reason for the commercial line SBA-25 showing a positive GCA value for GY, in contrast to SBA-12, (which showed a negative value), was due to possible differences in obtention periods, around the '90s (13) and '50s (28), respectively. Thus, in the first line, more recently created, greater recombination and genetic advance were achieved. The agronomic traits showing predominant general combining ability, respond to present additive genes. Zewdie *et al.* (2001), working with hot pepper, and Khan *et al*. (2009) with sunflower, suggested that for this type of predominating genes, recurrent reciprocal selection allows good genetic improvement.

SGW: specific grain weight (kg hl^{-1}); DF: days to flowering; PH: plant height (cm); EL: excertion length (cm); PL: panicle length (cm); and GY: grain yield (kg ha^{-1}). *, **: Significant at 0.05 and 0.01% probability levels, respectively.

SGW: peso específico del grano (kg hl^{-1}); DF: días a floración; PH: altura de planta (cm); EL: longitud de excersión (cm); PL: longitud de panícula (cm); y GY: rendimiento de grano (kg ha^{-1}). *, **: Significativo al nivel de 0,05 y 0,01% de probabilidad, respectivamente.

Table 2. Estimates of general combining ability (GCA) in lines for agronomic traits in INIFAP, Las Huastecas Experimental Station, Altamira, Municipality, Tamaulipas, México. 2018, Spring-Summer cycle.

Tabla 2. Valores estimados de los efectos de aptitud combinatoria general (ACG) en líneas, para las variables agronómicas. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

Lines	SGW (kg/hl^{-1})	DF (days)	PH (cm)	EL (cm)	PL (cm)	GY (kg/ha^{-1})
RB214A	-22.275	0.300	6.467**	0.250	0.992**	264.575**
RB225A	29.392**	3.300**	4.133**	2.375**	-0.300	346.117**
RB248A	6.933**	-0.158	2.800**	0.125	0.742**	185.283**
SBA-12	16.642**	-3.283	-10.950	-1.000	-2.217	-819.550
SBA-25	-30.692	-0.158	-2.450	-1.750	0.783**	23.575**

The GCA for GY (table 3), in the genotypes RB133, RB221 and RTx430, was highly significant and higher than commercial RTx437, which presented a negative response. For GSW, four out of eight testers presented positive and highly significant values of GCA.

SGW: specific grain weight (kg hl^{-1}); DF: days to flowering; PH: plant height (cm); EL: excertion length (cm); PL: panicle length (cm); and GY: grain yield (kg ha^{-1}). *, **: Significant at 0.05 and 0.01% probability levels, respectively.

SGW: peso específico del grano (kg hl^{-1}); DF: días a floración; PH: altura de planta (cm); EL: longitud de excersión (cm); PL: longitud de panícula (cm); y GY: rendimiento de grano (kg ha^{-1}). *, **: Significativo al nivel de 0,05 y 0,01% de probabilidad, respectivamente.

Table 3. Estimates of general combining ability (GCA) in testers for agronomic traits. INIFAP, Las Huastecas Experimental Station, Altamira, Municipality, Tamaulipas, México. 2018, Spring-Summer cycle.

Tabla 3. Efectos de aptitud combinatoria general (ACG) estimada para las variables agronómicas en los probadores. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

Tester	SGW (kg hl^{-1})	DF (days)	PH (cm)	EL (cm)	PL (cm)	GY (kg ha^{-1})
RB128	9.542**	-0.292	-10.292	0.792**	-0.683	-213.792
RB133	4.475**	0.042	4.508**	0.525*	2.383**	811.275**
RB135	-38.992	2.708**	-4.358	-2.542	-0.683	-328.192
RB221	30.342**	3.108**	40.375**	-2.075	1.517**	1678.808**
RB256	-12.458	-2.292	-8.625	0.258	-0.150	-805.925
RB373	17.608**	0.242	-7.692	2.458**	-1.750	-1452.525
RTx430	-9.258	-1.025	-6.692	-0.142	0.050	388.475**
RTx437	-1.258	-2.492	-7.225	0.725**	-0.683	-78.125

Specific combining ability (SCA) for GY in table 4 (page 17), shows 17 experimental highly significant hybrids (p≤0.01%), while INIFAP´s commercial hybrids, RB-3030, RB-4000 and RB-Huasteco, presented a negative response. Positive effects of SCA, indicate dominant and epitasis genes. On the other hand, genotypes presenting negative values show parental unfavorable combinations. The SCA importance for sorghum GY has already been reported (3, 23, 24). For SGW, half the hybrids showed highly significant differences, indicating non-additive gene importance. Regarding PH, 11 hybrids resulted highly significant and 11 significant.

This turns favorable for hybrid selection considering suitable plant height. DF, PL and EL, showed few significant differences.

Table 5 (page 18) shows proportional line contribution and lines x tester for six agronomic traits. Lines played an evident role in SGW (41.11%) and DF (47.24%), indicating maternal predominance. Testers showed more influence in DF (40.14), PH (68.65%), EL (35.49%), PL (35.71%) and GY (58.88%). Previously, Mohammed (2009), found the same results in forage sorghum for lines in green fodder and dry fodder production, while Pataki *et al*. (2007) found greater line influence on plant height.

A positive and significant correlation was found for grain yield (p≤0.001) and panicle length (figure 1, page 18), in coincidence with Makanda *et al*. (2010) and Bunphan *et al*. (2015). Williams *et al*. (2015) mentioned that the highest-yielding hybrids had higher panicle lengths. In addition, a significant correlation (p≤0.001) was also found for grain yield in kg ha^{-1} and plant height (Sarvari and Behesthi, 2012). This significant correlation between PH and PL is given by the fact that taller plants and greater panicle length are correlated with higher grain yield. A positive correlation was also found between SGW, PH, PL and GY.

Table 4. Estimation effects of specific combining ability (SCA) in agronomic traits. INIFAP, Las Huastecas Experimental Station, Altamira, Municipality, Tamaulipas, Mexico. 2018, Spring-Summer cycle.

Tabla 4. Estimación de los efectos de aptitud combinatoria específica (ACE) en las variables agronómicas. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

Crosses	SGW (kg hl^{-1})	DF (days)	PH (cm)	EL (cm)	PL (cm)	GY (kg ha^{-1})
RB214A*RTx430	-37.325	0.233	-12.933	2.350**	-2.592	136.358**
RB225A*RTx430	8.342**	-1.767	-0.600	1.558	1.033	745.483**
RB248A*RTx430	35.467**	-1.308	-0.600	-1.858	-1.008	-269.350
SBA12*RTx430[1]	-22.908	0.483	12.483**	-1.733	0.283	-568.183
SBA25*RTx430[2]	16.425**	2.358*	1.650	-0.317	2.283**	-44.308
RB214A*RTx437	-19.992	-0.967	-6.733	-0.183	0.808	314.292**
RB225A*RTx437	30.342**	0.033	3.600**	-1.308	-0.567	-437.583
RB248A*RTx437	-18.533	0.492	5.267**	-0.725	0.725	410.917**
SBA12*RTx437	11.758**	0.617	-6.317	2.067*	-0.650	-223.250
SBA25*RTx437[3]	-3.575	-0.175	4.183**	0.150	-0.317	-64.375
RB214A*RB128	-4.125	-0.167	-3.667	0.083	-0.192	-566.042
RB225A*RB128	10.875**	1.833*	4.333**	2.958**	0.433	-5.250
RB248A*RB128	6.667**	-0.042	-1.667	-1.125	1.058	511.250**
SBA12*RB128	0.958	-0.917	2.417*	-1.000	0.017	704.750**
SBA25*RB128	-14.375	-0.708	-1.417	-0.917	-1.317	-644.708
RB214A*RB133	20.942**	-0.500	1.867*	1.017	0.742	-184.108
RB225A*RB133	13.275**	-1.167	0.200	-1.108	0.700	1415.350**
RB248A*RB133	1.067	-0.375	-3.800	-0.192	0.325	-1180.817
SBA12*RB133	-39.308	2.417*	-3.717	0.600	-1.717	-596.650
SBA25*RB133	4.025**	-0.375	5.450**	-0.317	-0.050	546.225**
RB214A*RB135	16.408**	0.833	37.733**	0.083	1.475	1000.692**
RB225A*RB135	-16.592	0.833	-17.600	-2.042	-1.900	-279.517
RB248A*RB135	-6.133	-1.042	2.067*	0.875	2.392**	231.317**
SBA12*RB135	14.825**	-1.583	-6.517	1.000	0.017	534.150**
SBA25*RB135	-8.508	0.958	-15.683	0.083	-1.983	-1486.642
RB214A*RB221	13.742**	0.767	0.000	0.950	-1.392	-427.642
RB225A*RB221	-30.258	0.100	-4.667	-2.842	0.900	-402.183
RB248A*RB221	-27.133	1.892*	1.333	0.075	-2.142	-514.350
SBA12*RB221	24.158**	-2.317	-2.917	0.533	0.817	-121.183
SBA25*RB221	19.492**	-0.442	6.250**	1.283	1.817*	1465.358**
RB214A*RB256	-9.458	-0.500	-4.333	-3.383	0.942	77.758**
RB225A*RB256	8.875**	-0.500	2.667**	0.492	-1.100	-381.783
RB248A*RB256	-2.000	0.958	-3.000	3.075**	-0.142	-297.950
SBA12*RB256	26.958**	1.417	6.083**	0.867	0.817	265.883**
SBA25*RB256	-24.375	-1.375	-1.417	-1.050	-0.517	336.092**
RB214A*RB373	19.808**	0.300	-11.933	-0.917	0.208	-351.308
RB225A*RB373	-24.858	0.633	12.067**	2.292**	0.500	-654.517
RB248A*RB373	10.600**	-0.575	0.400	-0.125	-1.208	1108.983**
SBA12*RB373	-16.442	-0.117	-1.517	-2.333	0.417	4.483**
SBA25*RB373	10.892**	-0.242	0.983	1.083	0.083	-107.642

SGW: specific grain weight (kg hl^{-1}); DF: days to flowering; PH: plant height (cm); EL: excertion length (cm); PL: panicle length (cm); and GY: grain yield (kg ha^{-1}). *, **: Significant at 0.05 and 0.01 % probability levels, respectively. [1]RB-3030. [2]RB-Patrón. [3]RB-Huasteco.

SGW: peso específico del grano (kg hl^{-1}); DF: días a floración; PH: altura de planta (cm); EL: longitud de excersión (cm); PL: longitud de panícula (cm); y GY: rendimiento de grano (kg ha^{-1}). *, **: Significativo al nivel de 0,05 y 0,01% de probabilidad, respectivamente.

Table 5. Contribution of Lines, testers, and lines x testers to the total variance in agronomic traits. INIFAP, Las Huastecas Experimental Station, Altamira, Municipality, Tamaulipas, México. 2018, Spring-Summer cycle.

Tabla 5. Contribución de las líneas, probadores y líneas x probadores en la varianza para las variables agronómicas estudiadas. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

	Contribution (%)		
Trait	**Lines**	**Testers**	**Line x tester**
Specific grain weight	41.11	30.36	28.51
Days to flowering	47.24	40.14	12.61
Plant height	10.50	68.65	20.79
Excertion length	30.31	35.49	34.19
Panicle length	32.77	35.71	31.15
Grain yield	12.90	58.88	28.21

SGW: specific grain weight (kg hl^{-1}); DF: days to flowering; PH: plant height (cm); EL: excertion length (cm); PL: panicle length (cm); and GY: grain yield (kg ha^{-1}). *, **, ***: Significant at 0.05, 0.01 and 0.001 % probability levels, respectively.

SGW: peso específico del grano (kg hl-1); DF: días a floración; PH: altura de planta (cm); EL: longitud de excersión (cm); PL: longitud de panícula (cm); and GY: rendimiento de grano (kg ha-1); *,**,***: Significativo al nivel de 0,05 y 0,01% de probabilidad, respectivamente.

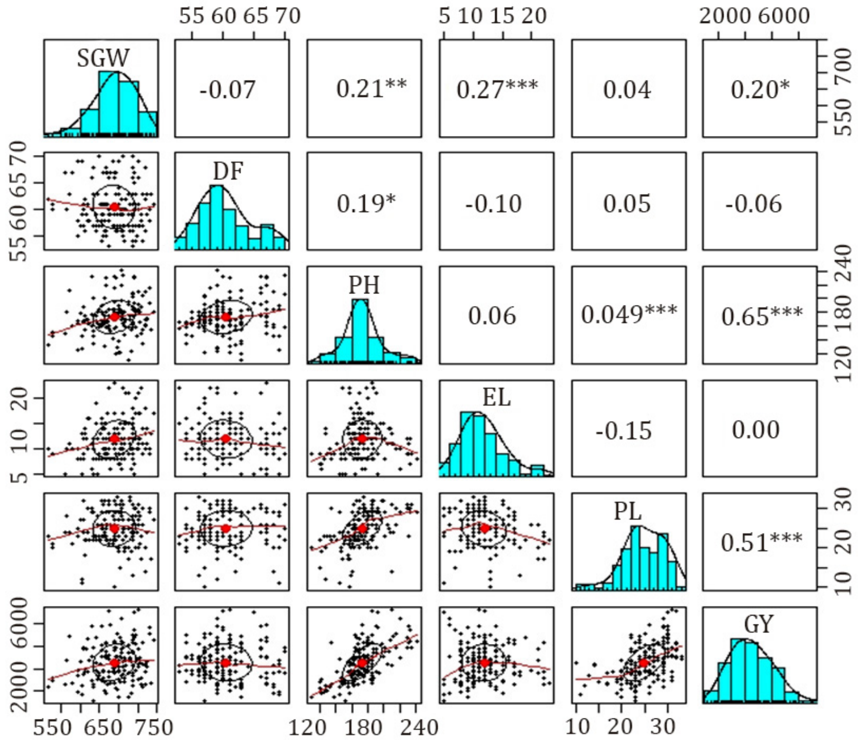

Figure 1. Correlation coefficients between agronomic variables for hybrids and sorghum parents. INIFAP, Las Huastecas Experimental Station, Altamira Municipality, Tamaulipas, México. 2018, Spring-Summer cycle.

Figura 1. Coeficientes de correlación entre las variables agronómicas para híbridos y progenitores de sorgo. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

Table 6 shows the mean values of the best experimental and commercial hybrids for grain yield, ranging from 3941 to 8108 kg ha^{-1}, where SBA25xRB221 experimental hybrid showed more than 8,000 kg ha^{-1}. In this regard, Williams *et al.* (2015), conducted evaluations of experimental and commercial hybrids during three years in INIFAP, obtaining maximum grain yields of 6400 kg ha^{-1}, under favorable soil moisture and management conditions. Table 6 shows that 11 experimental hybrids resulted statistically equal ($p<0.05$) in grain yield to the commercial controls: Pioneer® P83P27 and P85P20, RB-Patrón and RB-Huasteco. Within this group, two experimental hybrids, SBA25xRB221 and RB225AxRB133, were superior to RB-3030 and seven other experimental hybrids. In both cases, parental lines showed highly significant values for GCA (table 2, page 15, and table 3, page 16) while the hybrids showed high SCA (table 4, page 17). Williams *et al.* 2015 indicated that tall plant hybrids usually present lodging problems, and difficult mechanical harvesting. Therefore, we considered the experimental hybrid SBA25xRB221, 225 cm high, not suitable for commercial planting. Hybrid height depends on parental GCA and hybrid SCA height (table 2, page 15; table 3, page 16, and table 4, page 17). We concluded that the experimental hybrid RB225AxRB133 showed the best grain yield and plant height combination.

For grain yield, some experimental lines, testers and hybrids turned out superior to commercial ones. For grain yield, specific grain weight and plant height, additive genes as well as dominance and epistasis genes resulted important. The general combining ability in lines was proportionally higher in genotype number for specific grain weight, plant height and grain yield than testers. Results indicated that experimental lines and testers are promising for hybrid development and breeding.

Table 6. Sorghum hybrids and tested agronomic traits. INIFAP, Las Huastecas Experimental Station, Altamira Municipality, Tamaulipas, México. 2018, Spring-summer cycle.

Tabla 6. Híbridos de sorgo y variables agronómicas evaluadas. INIFAP, Campo Experimental las Huastecas, Municipio de Altamira Tamaulipas, México, ciclo primavera-verano 2018.

GENEALOGY	GY (kg ha^{-1})	SGW (kg hl^{-1})	DF (days)	PH (cm)	EL (cm)	PL (cm)
SBA25xRB221	8108 a	708 a-e	62 a-h	225 ab	10 a-d	30 a
RB225AxRB133	7513 ab	736 a-d	62 a-h	190 c-f	14 a-d	29 a-c
RB225AxRB221	6563 a-c	718 a-e	66 a-d	221a-c	10 a-d	28 a-c
RB214AxRB221	6456 a-c	711 a-e	64 a-h	228 a	11 a-d	27 a-d
RB225AxTx430	6421 a-d	717 a-e	60 a-h	178 d-j	16 a-d	27 a-d
SBA25xRB133	6322 a-e	667 a-f	59 a-h	188 c-h	11 a-d	29 ab
RB248AxRB221	6290 a-e	699 a-e	65 a-g	225 ab	10 a-d	26 a-d
RB214AxRB135	5878 a-f	644 a-f	64 a-h	221 a-c	10 a-d	28 a-c
PioneerP83P27 (C)	5834 a-g	745 a-d	54 h	155 h-n	12 a-d	25 a-d
RB214AxRB133	5832 a-g	692 a-f	60 a-h	194 b-e	14 a-d	30 a
PioneerP85P20 (C)	5813 a-g	708 a-e	68 ab	189 c-g	10 a-d	26 a-d
RB214AxTx430	5730 a-g	620 c-f	59 a-h	168 e-l	15 a-d	25 a-e
SBA12xRB221	5679 a-g	760 ab	57 b-h	207a-d	10 a-d	26 a-d
RB-Patrón (C)	5308 a-h	665 a-f	61 a-h	173 e-k	10 a-d	29 ab
RB-Huasteco (C)	4822 a-h	653 a-f	57 c-h	175 d-k	11 a-d	26 a-d
RB-3030 (C)	3941 c-h	673 a-f	56 d-h	176 d-k	9 a-d	24 a-e

GY: grain yield (kg ha^{-1}), SGW: specific grain weight (kg hl^{-1}); DF: days to flowering; PH: plant height (cm); EL: excertion length (cm); PL: panicle length (cm); (C) Controls. Different letters (a, b, c) in each trait and within the same group denote statistical significance (Tukey; p=0.05).

GY: rendimiento de grano (kg ha^{-1}); SGW: peso específico del grano (kg hl^{-1}); DF: días a floración; PH: altura de planta (cm); EL: longitud de excersión (cm); PL: longitud de panícula (cm); (C): Controles. Literales diferentes (a,b,c) en cada variable y dentro del mismo grupo denotan significancia estadística (Tukey; p=0,05).

Conclusions

The results showed that general combining ability (GCA) and specific combining ability (SCA) were important for grain yield, grain-specific weight and plant height. For grain yield, the experimental lines RB214A, RB225A and RB248A, and the testers RB133 and RB221, resulted superior in GCA than commercial ones. Seventeen experimental hybrids were found to have better SCA than INIFAP commercial hybrids.

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Physical and chemical characterization of yacon (*Smallanthus sonchifolius*) roots cultivated with different doses of potassium fertilization

Caracterización física y química de raíces de yacón (*Smallanthus sonchifolius*) cultivadas con diferentes dosis de fertilización potásica

Amanda Dutra de Vargas [1*], Fábio Luiz de Oliveira [1], Luciano José Quintão Teixeira [2], Mateus Oliveira Cabral [1], Lidiane dos Santos Gomes Oliveira [1], Joab Luhan Ferreira Pedrosa [1]

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Abstract

The already marked tendency for functional food consumption, low in calories, and with biologically active properties, has been increasing. In this scenario, yacon tuberous roots, with high levels of nutraceutical fructooligosaccharides, gain importance. However, these nutraceutical properties depend on fertilization management. Thus, our study aims to evaluate different doses of potassium fertilization on the physicochemical characteristics of yacon roots. The experimental design consisted of randomized blocks, with 4 replications and 5 treatments: four doses of potassium fertilization (50%; 100%; 150%; 200%) of the reference value, corresponding to 178.7 $kg.ha^{-1}$; 357.4 $kg.ha^{-1}$; 536.1 $kg.ha^{-1}$; 714.8 $kg.ha^{-1}$ of potassium chloride – (KCl), and a control (soil without fertilization). The chemical features evaluated were pH, total titratable acidity, soluble solids, conductivity, turbidity, moisture and ashes. The texture profile was analyzed through toughness, adhesiveness, cohesiveness, chewability index, elasticity, and gooeyness. Most of the variables were influenced by potassium soil fertilization. Only turbidity, conductivity and gumminess showed no response to the applied doses, not fitting the tested models. Potassium fertilization improved both chemical (higher levels of soluble solids and less acidity) and physical characteristics (less hardness, chewability, cohesiveness, and adhesion) of yacon tuberous roots, bringing greater quality to the final product.

Keywords
Smallanthus sonchifolius • fructooligosaccharides • functional food • tuberous roots

1 Universidade Federal do Espírito Santo. Departamento de Agronomia. Centro de Ciências Agrárias e Engenharias. Alegre. Espírito Santo. Brasil.
* amandadvargas@hotmail.com
2 Universidade Federal do Espírito Santo. Departamento de Engenharia de Alimentos. Centro de Ciências Agrárias e Engenharias. Alegre. Espírito Santo. Brasil.

Resumen

La tendencia en el consumo de alimentos funcionales, con pocas calorías y con propiedades biológicamente activas, ha ido en aumento. El consumo de raíces tuberosas de yacón se adapta a este concepto por los altos niveles de fructooligosacáridos, lo que le confiere propiedades nutracéuticas. Sin embargo, estas propiedades pueden modularse mediante el manejo de la fertilización en el cultivo de esta planta. Así, nuestro estudio tiene como objetivo evaluar diferentes dosis de fertilización potásica en las características fisicoquímicas de las raíces de yacón. El diseño experimental utilizado fue de bloques al azar, con 4 repeticiones y 5 tratamientos: cuatro dosis de fertilización potásica (50%; 100%; 150%; 200%) del valor de referencia, correspondiente a 178,7 $kg.ha^{-1}$; 357,4 $kg.ha^{-1}$; 536,1 $kg.ha^{-1}$; 714,8 $kg.ha^{-1}$ de cloruro de potasio - (KCl), y un testigo (suelo sin fertilizar). Las características químicas evaluadas fueron pH, acidez total titulable, sólidos solubles, conductividad, turbidez, porcentaje de humedad y cenizas. El perfil de textura se elaboró analizando la tenacidad, la adhesividad, la cohesión, el índice de masticabilidad, la elasticidad y la pegajosidad. La fertilización con potasio mejoró las características químicas (mayores niveles de sólidos solubles y menor acidez) y las características físicas (menor dureza, masticabilidad, cohesión y adherencia) de las raíces tuberosas de yacón, aportando mayor calidad al producto final.

Palabras clave

Smallanthus sonchifolius • fructooligosacáridos • comida functional • raíces tuberosas

Introduction

The search for foods with low carbohydrates and greater amounts of antioxidants and vitamins, *i.e.* with biologically active value, has increased (11). In this context, yacon (*Smallanthus sonchifolius*) an Asteraceae of Andean origin, gains importance for its tuberous roots with high concentrations of inulin and fructooligosaccharides (FOS) (4).

Yacon is considered a prebiotic food. Several immunostimulatory characteristics promote antimicrobial, anti-inflammatory, and antioxidant activity (51). It also acts in the regulation of appetite (45), increasing mineral availability (23), positively modulating the immune system (52), in the hypolipidemic effect (33) and preventing diseases such as diabetes and cancer (40).

The tuberous roots of yacon are sweet and slightly crunchy, resembling fruits such as apple, pear, watermelon, and melon, mostly consumed in fresh. However, other forms of consumption have also been studied (24).

Chemical characteristics, such as pH, soluble solids, and total titratable acidity, together with texture, flavor, and appearance constitute some of the quality attributes leading consumers to accept or reject a given food (28). Variations in the physical and chemical characteristics of yacon tuberous roots have been studied regarding cultivation and processing (46, 50). Thus, studying techniques that improve its production system increasing quality, turns essential for a successful production.

In plant nutrition, potassium is considered an essential nutrient. It participates in enzymatic activation, protein formation, photosynthesis (21), regulation of osmotic pressure, and opening and closing of stomata (48). It is also associated with root size, shape, texture, color, flavor, acidity, nutrient transport resistance, nutritional value (38), and even market value (12). Considering this, optimizing the use of potassium constitutes an important tool in crop management, and since information related to this matter is still scarce, our study aims to evaluate the physicochemical characteristics of yacon tuberous roots as a function of different doses of potassium fertilization.

Material and methods

The experiment was located in the municipality of Alegre, in the State of Espírito Santo, Brazil, (20°47’1” S, 41°36’56” W, 680 m a. s. l.). Precipitation, relative humidity, and monthly temperature averages were obtained by automatic meteorological stations close to the experiment, using Incaper in Iúna, Espirito Santo, Brazil (20°21' S, 41°33' W, 758 m a. s. l. (figure 1). Average monthly temperatures fluctuated from 17.79 to 21.43°C, and rainfall summed 638 mm during field experiments.

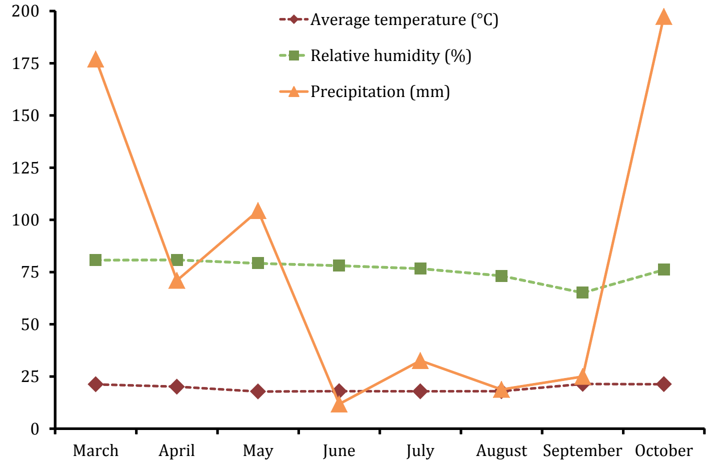


Figure 1. Monthly averages of precipitation, relative humidity, and temperature during the experimental period. Alegre-ES, 2020.

Figura 1. Promedios mensuales de precipitación, humedad relativa y temperatura durante el período del experimento. Alegre-ES, 2020.

Soil samples were collected and subjected to laboratory analysis, showing the following chemical characteristics: water pH 4.80 , P 3.95 mg dm^{-3} , K 42.00 mg dm^{-3} , Ca 0.68 $cmol_c$ dm^{-3}, Mg 0.22 $cmol_c$ dm^{-3} , Al 1.00 $cmol_c$ dm^{-3} , exchangeable bases,1.01 $cmol_c$ dm^{-3} cation exchange capacity 2.01 $cmol_c$ dm^{-3} and base saturation index 8.89%.

Soil preparation was done by plowing and harrowing at a depth of 40 cm. Liming was performed using dolomitic limestone with 96% PRNT increasing base saturation to 70%, with a period of 60 days for planting. For the propagation of yacon, 30 grams rhizophores with three to four buds were used, as recommended by Pedrosa *et al.* (2020) and planted individually on ridges with a spacing of 1.0 m × 0.5 m, as recommended by Carvalho *et al.* (2020).

The experiment was conducted in a randomized complete block design, with four replications of four different doses of potassium fertilization: 50%; 100%; 150%, and 200% of the reference value (26); and a control (soil without fertilization). The experimental plot consisted of three lines with three plants providing nine useful plants for evaluations, bordered by 2 rows and 2 extra plants. The reference value considered, at the end of 210 days after planting, was 106.8 kg. ha^{-1} of K in yacon plants (26). Final dose estimation, considering K_2O, resulted128.65 kg. ha^{-1}. A recovery efficiency of 60% for K was also considered (43). Thus, potassium chloride (KCl) doses applied were: 178.7 kg. ha^{-1}; 357.4 kg. ha^{-1}; -536.1 kg. ha^{-1} and 714.8 kg. ha^{-1}, equivalent to 50, 100, 150 and 200%. Two applications were made in coverage. The first application was performed when 80% of the plants emerged, with the first pair of leaves open (80 days after planting) and the second application was done 30 days later (110 days after planting).

Nitrogen and phosphate fertilization, were added with 382.2 kg.ha^{-1} of urea and 422.6 kg.ha^{-1} of simple superphosphate (26), achieving 172 kg.ha^{-1} for N and 33.2 kg.ha^{-1} for P (converted to 76.07 kg of P_2O_5). Phosphate fertilization was performed at planting while nitrogen fertilization was performed in coverage along with potassium. During the whole experimental period, irrigation was done by sprinkling while weeds were manually controlled.

At the end of the experiment, 210 days after planting, the plants were harvested and the roots separated and taken to the Food Chemistry Laboratory of the CCAE/UFES for chemical analyzes such as pH, soluble solids, total acidity, conductivity, turbidity, humidity, and ashes. A sample of these roots was separated for physical analysis, at the Food Science and Technology Laboratory (CCAE/UFES), where the following parameters were observed: toughness, adhesiveness, cohesiveness, index of chewability, elasticity, and gooeyness.

For chemical analysis, the roots were cut, washed, dried, and taken to a centrifuge to extract the juice, then filtered with filter paper for later evaluations. Juice direct readings of pH, electrical conductivity, and soluble solids were conducted.

Total titratable acidity was obtained by a diluted sample of the juice (5 mL of juice + 50 mL of distilled water), titrated with 0.1 mol L^{-1} NaOH solution, using phenolphthalin, according to Instituto Adolfo Lutz (2008). Acidity was calculated as a function of malic acid, the most expressive acid in yacon. Turbidity, in 10 mL of juice + 40 mL of distilled water was assessed using a turbidimeter.

Subsequently, roots from each treatment were grated and a sample was incinerated in the muffle at 550°C, until constant weight, obtaining ashes. For humidity, another set of samples was oven-dried at 105°C. Finally, root samples were standardized at 2 cm thickness and 4 cm diameter for texture analysis. Parameters were determined using the Brookfield CT3 texturometer and the TA39 needle probe, with a test speed of 2 mm s^{-1}. The target distance for drilling was set at 5 mm.

Linear regression models were tested for data processing. The analysis was performed based on average values for each treatment (four repetitions). The sum of squares, R^2 (coefficient of determination), and significance of regression coefficients constituted model parameters. Statistical analyses were performed with the open code software R (39).

Results and discussion

Turbidity and conductivity did not fit any model. The doses used did not influence root chemical variability (figure 2 AB).

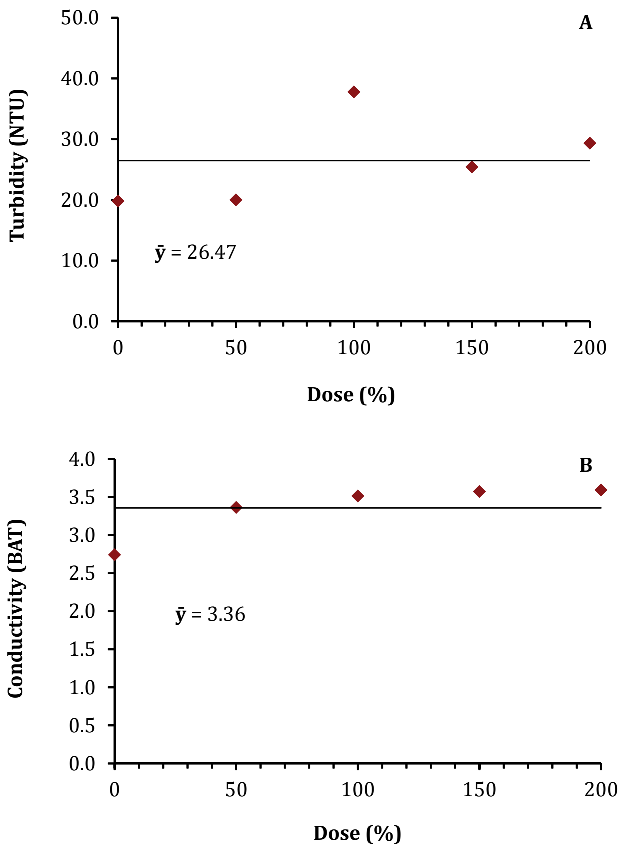


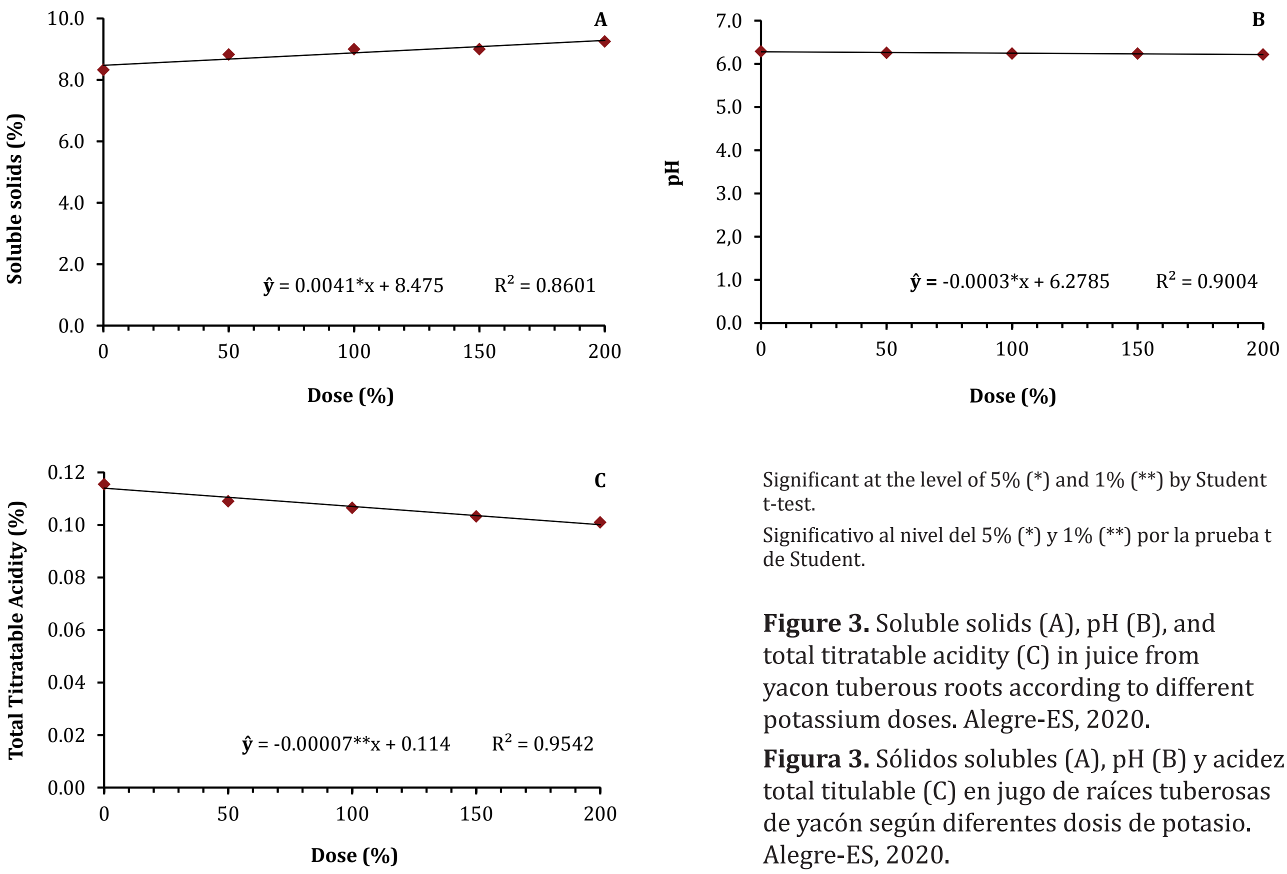
Figure 2. Turbidity (A) and electrical conductivity (B) in juice of yacon tuberous roots, according to different potassium doses. Alegre-ES, 2020.

Figura 2. Turbidez (A) y conductividad eléctrica (B) en el jugo de raíces tuberosas de yacón, según las diferentes dosis de potasio. Alegre-ES, 2020.

No change in juice turbidity means that the tested potassium doses did not influence insoluble solids in suspension (proteins, pectin, lipids, cellulose, and hemicellulose) in yacon roots. Insoluble solids remain in suspension after cell rupture during juice preparation (20). Juice turbidity is essential for market acceptance (8), thus, the non-alteration of this characteristic is a positive result evidencing that potassium fertilization would not cause quality losses.

Likewise, conductivity did not change, possibly due to the non-variation in turbidity, since the first is influenced by several factors, such as electrolyte concentration and temperature (27). Conductivity reflects solutions ionic behavior, and according to Icier and Ilicali (2005), can be altered by the content of insoluble solids. Several studies point out this relationship. Some authors evaluated the effect of suspended-particle size in mango juice and observed higher electrical conductivity in particle-free samples, noting that, probably, intermediate-sized particles hinder ionic movement (54). A similar result was found by Pelacani and Vieira (2003), also in mango juice and Palaniappan and Sastry (1991), in carrot juice. Both studies showed that conductivity is higher in solutions with smaller insoluble particles. Regarding the direct influence of potassium doses, Gurgel *et al.* (2010) evaluating postharvest quality of melons, obtained similar results for juice conductivity, in which regression analyzes did not fit the tested models.

Soluble solids, pH, and total titratable acidity achieved linear model fitting. Soluble solids content showed a slight increase, reaching 10% when comparing the highest tested dose with the unfertilized treatment (figure 3A). The pH, on the other hand, showed a slight decrease, although more pronounced for total acidity, which decrease by 17% with the highest potassium dose (figure 3 BC). Increased total soluble solids with the highest availability of potassium occur as a result of a potassium-mediated favored carbohydrates formation and translocation (22). Potassium influences plant water transport stimulating solute storage in organs such as seeds, tubers, roots, and fruits (25, 38).



Significant at the level of 5% (*) and 1% (**) by Student t-test.

Significativo al nivel del 5% (*) y 1% (**) por la prueba t de Student.

Figure 3. Soluble solids (A), pH (B), and total titratable acidity (C) in juice from yacon tuberous roots according to different potassium doses. Alegre-ES, 2020.

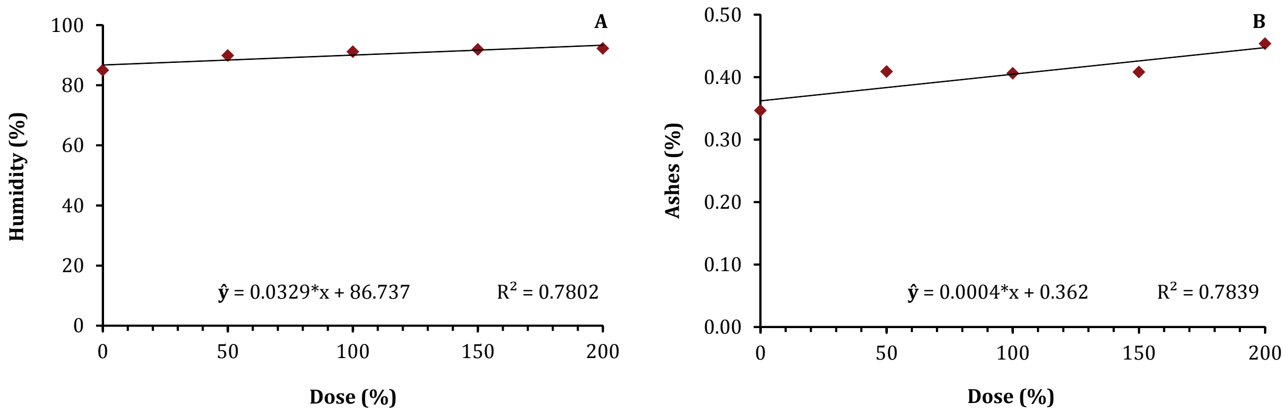
Figura 3. Sólidos solubles (A), pH (B) y acidez total titulable (C) en jugo de raíces tuberosas de yacón según diferentes dosis de potasio. Alegre-ES, 2020.

The results show a contribution of potassium fertilization for yacon roots quality evidenced by increased sugar content (main component of soluble solids), vitamin C, other acids, and some pectins, as previously mentioned (7), rising the possibility for this product´s market better acceptance.

The observed subtle decrease in acidity (pH and total acidity) may have occurred after a greater conversion of sugars (higher levels of soluble solids) and degradation of organic acids (19, 35), possibly after accelerated root maturation, given greater availability of potassium. Similar results were observed by Barreto *et al.* (2020) with peach, Delgado *et al.* (2004) with grapes, and Veloso *et al.* (2001) with pineapple, who reported fruit ripening anticipation, favored by increasing available potassium.

In fruit ripening, higher sugar levels cause sharp neutralization of organic acids, making the fruit less acidic and sweeter (14). This possibility was also pointed out by Silva *et al.* (2018) for yacon roots, after observing a reduction in acidity at an established stage of life-cycle ending, then considered as the proper harvest time for sweeter roots, an essential characteristic for palatability and better market acceptance.

Regarding humidity and ashes, an increasing linear fit showed that for the highest potassium dose (200%) both variables increased 8 and 22%, respectively (figure 4).



Significant at 5% (*) by Student t-test.

Significativo al nivel del 5% (*) según la prueba t de Student.

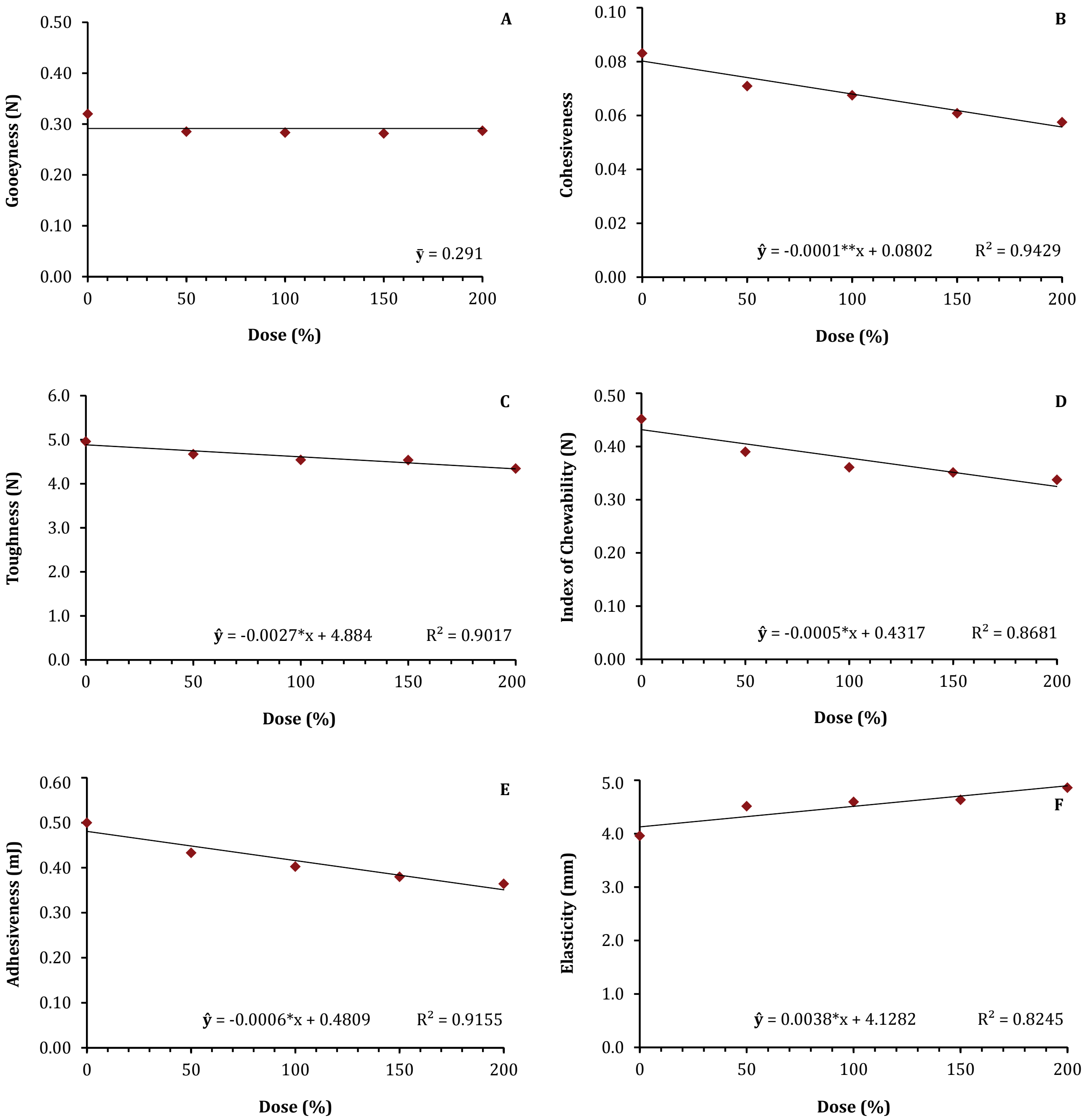
Figure 4. Humidity (A) and ashes (B) in yacon tuberous roots, according to increasing potassium doses (Alegre-ES, 2020).

Figura 4. Humedad (A) y cenizas (B) en raíces tuberosas de yacón, según las diferentes dosis de potasio (Alegre-ES, 2020).

This increased humidity in yacon roots means high water content and low energetic food, both high-quality standards (41). Humidity may vary depending on a range of interactive factors, including field conditions, planting, harvest, and fertilization (34). Similar results in potato tubers obtained by Quadros *et al.* (2009) pointed out that the highest potassium dose provided higher percentages of humidity. However, considering post-harvest conservation, significant higher moisture contents may cause greater difficulty in preserving the product (44, 47). Still, the resulted increase (8% for the highest dose compared to the control) turns irrelevant, as with fast and adequate storage (dehydration preventing conditions) (49), no significant losses should complicate commercialization.

The higher levels of ashes are related to increasing root mineral contents after potassium fertilization. This was already observed by Quadros *et al.* (2009) with common potatoes. Additionally, Oliveira *et al.* (2015) state the importance of potassium supply, through balanced nutrition, since product quality (tuber, rhizomes, and tuberous roots), may vary according to the performed fertilization.

Regarding texture analysis, only gooeyness values resulted independent of potassium doses. For cohesiveness, toughness, index of chewability, and adhesiveness, increasing potassium doses resulted in decreasing values. Elasticity, in turn, increased with increasing doses (figure 5).



Significant at 5% (*) and 1% (**) by Student *t*-test.
Significativo al nivel del 5% (*) y del 1% (**) según la prueba t de Student.

Figure 5. Gooeyness (A), cohesiveness (B), toughness (C), index of chewability (D), adhesiveness (E), and elasticity (F) of yacon tuberous roots, as a function of increasing potassium doses (Alegre- ES, 2020).

Figura 5. Pegajosidad (A), cohesión (B), tenacidad (C), índice de masticabilidad (D), adhesividad (E) y elasticidad (F) de las raíces tuberosas de yacón, en función de diferentes dosis de potasio (Alegre- ES, 2020).

Gooeyness is related to the force required to disintegrate food, by dissociating its mass. For Bolzan and Pereira (2017), it is associated with toughness and cohesiveness. However, in this case, despite the linear decrease in cohesiveness and hardness with the increasing potassium doses, no gooeyness alteration was evidenced in the analyzed yacon tuberous roots. That is, yacon roots can withstand rupture (cohesiveness measure), and demand less strength to be obstructed (toughness measure) (1). In this sense, in a prior sensory analysis with the application of potassium doses, the roots became softer, requiring less strength (25% less with the highest dose of potassium) to be squeezed between the molar teeth at the first bite (14). The same was noticed for chewability (around 24% reduction of needed strength with the highest dose of potassium) (9). These results evidence gains in sensory quality of yacon roots fertilized with potassium.

Decreased adhesion occurred after increasing humidity, which, according to Rahman and Al-Farsi (2005) are inversely proportional characteristics. In sensory terms, increasing potassium doses, eases food ingestion, due to lower adherence strength (reduction of around 28% with the highest dose of potassium), increasing sensory quality.

The resulting root-increased elasticity was expected since this characteristic is inversely proportional to hardness, cohesiveness, and chewability (42). This greater elasticity (18% in the maximum dose) contributed to root quality.

Finally, texture constitutes an important factor and quality criterion for the sensory acceptance of food (6). Thus, the results obtained show that potassium fertilization improves both physical and chemical characteristics of yacon roots.

Conclusion

Potassium fertilization improved both physical and chemical characteristics of yacon roots, The best results were observed with the maximum applied dose (357.4 kg.ha^{-1} of KCl).

With the application of the maximum dose, highest levels of soluble solids (9.25%), moisture (92.24 %), ash (0.454 %) and elasticity (4.86 mm) and lower acidity (0.101 %) and pH (6.22) were achieved, in addition to lowest chewability indexes (0,337 N), hardness (4,35 N), cohesiveness (0,058) and stickiness (0,364 mJ). Such results add greater value to the final quality of the roots.

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Sowing date effects on yield of three winter forage crops in the northern oasis of Mendoza

Efecto de la fecha de siembra en el rendimiento de tres verdeos invernales en el oasis norte de Mendoza

Alejandra Bertona, Cecilia Rebora, Leandra Ibarguren*

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Abstract

The increasing livestock farming in the province of Mendoza requests the acquisition of further knowledge on winter forage crops, annual grasses that produce a large volume of good quality biomass in a short time. The most widely used winter forage crops in Argentina are oats, rye and barley. To assess forage productivity in the northern oasis of Mendoza, an experimental factorial design combined 2 sowing dates (March 21, 2019, and April 29, 2019) and three winter forage crops: oats (Blanca Cristal INTA), rye (Lisandro INTA) and barley (Alicia INTA). Results showed no interaction between sowing dates and forage species. March sowing date was 27% more productive and offered a longer grazing period than April sowing (more than five months in March sowing and more than one month in April sowing). Oat showed the highest yield, differing significantly from rye and barley.

Keywords
Avena sativa • *Secale cereale* • *Hordeum vulgare*

Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Alte. Brown 500. Chacras de Coria. Mendoza. Argentina. M5528AHB.
* libarguren@fca.uncu.edu.ar

Resumen

La intensificación de la producción animal en la provincia de Mendoza requiere ampliar el estudio de recursos forrajeros. Los verdeos de invierno son gramíneas anuales que producen gran volumen de forraje de buena calidad, y son importantes en las cadenas forrajeras desde comienzos de otoño hasta avanzada la primavera. En Argentina, los verdeos invernales más utilizados son: avena, centeno y cebada. En el oasis norte de Mendoza, se planteó un experimento factorial combinando 2 fechas de siembra (21 de marzo de 2019 y 29 de abril de 2019) y los tres verdeos invernales: avena (Blanca Cristal INTA), centeno (Lisandro INTA) y cebada (Alicia INTA). No hubo interacción entre fechas de siembra y especies de verdeos invernales. La primera fecha de siembra resultó un 27% más productiva y su ventana de aprovechamiento fue mayor que en la segunda; resultando de más de cinco meses en la siembra de marzo y de un mes en la siembra de abril. La avena fue el cultivo con mayor producción, diferenciándose significativamente del centeno y la cebada.

Palabras clave
Avena sativa • *Secale cereale* • *Hordeum vulgare*

Introduction

Winter forage crops are annual grasses that produce a large volume of good-quality biomass in a short time. They play an important role in the forage chains from early autumn to late spring, when perennial cultivated or natural pastures show low forage availability (1, 9). Thus, given high seasonal production, winter forage crops are one efficient resource to be supplemented with perennial pastures, besides supporting stable forage production all year round (6). In addition, given they extend the grazing season through winter, they constitute an economically valuable alternative to the generally more expensive reserves (hay or silage).

According to the Ministerio de Agricultura, Ganadería y Pesca de Argentina, the most widely used winter forage crops in Argentina are oats (*Avena sativa*), barley (*Hordeum vulgare*) and rye (*Secale cereale*)(4). The average cultivated area during the 2020 campaign was: 1,405,535 ha of oats; 1,237,023 ha of barley and 667,809 ha of rye. These numbers represent the total planted area (hectares) regardless of whether they are harvested, grazed or two-fold purpose (8). Livestock migration to extra-pampean regions after the intrusion of agriculture has allowed Mendoza to develop intensive livestock farming activities.

The available information on forage crops in the irrigated oases of Mendoza is scarce. In the last years, some studies on alfalfa (12), corn and sorghum silages (5, 13) were reported. In this sense, determining the best sowing date is key for forage production. Records from different cultivated areas of the country show that, in general, early sowing of winter forage crops results in more productivity (1, 3, 10), However, species production potential may vary among environments (11). Given this situation and aiming to generate information on winter forage production in the northern oasis of the Mendoza Province, the following hypotheses were formulated: depending on the species (oats, rye, barley), early sowings (March) produce higher forage yields than late sowings (April).

Materials and methods

The experiment was conducted at the San Antonio farm of the Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, at 33°00’38” S and 68°52’28” W, on poorly developed alluvial silt loam soil. Mean annual temperature is 16.5°C, average relative humidity is 50% and average annual rainfall is 225 mm. Table 1 (page 34) shows monthly mean, minimum and maximum temperatures during the forage growing period:

Table 1. Monthly mean, minimum and maximum temperatures in degrees Celsius, from March to November 2019, Luján de Cuyo, Mendoza.

Tabla 1. Temperaturas medias, mínimas y máximas mensuales en grados centígrados, de marzo a Noviembre 2019, Luján de Cuyo, Mendoza.

	March	April	May	June	July	August	September	October	November
Mean T (°C)	17.6	16.1	10.6	7.6	7.1	9.1	11.9	15.1	21.4
Minimum T (°C)	11.3	9.1	5	0.7	0.1	1.1	4.2	7.7	14.7
Maximum T (°C)	24	23.1	16.2	14.6	15.2	17.2	19.6	22.4	28.2

The factorial experimental design was conducted in random blocks with 3 replications on 18 experimental plots: Two sowing dates (March 21, 2019, and April 29, 2019) for 3 winter forage crops: oats (Blanca Cristal INTA), rye (Lisandro INTA) and barley (Alicia INTA), and 3 replications per treatment. Table 2 shows crop management. Ten m^2 experimental plots (ten rows, 5m long and spaced 0.20 m), were sowed at a rate of 250 pl/m^2 (1, 3, 4, 9). Cuts were made at 40 cm forage height (ensuring appropriate plant anchorage and coverage between rows), leaving a remnant of 10 cm. Forage fresh weight was determined after each cut. Then, the percentage of oven-dried dry matter (% MS) was obtained at 60°C with forced-air circulation (on a 200 g sample). Finally, forage production per surface unit (kg DM/ha) was calculated. Each treatment was cut as many times as possible. At the end of the growing season, accumulated dry matter was determined.

Table 2. Crop management of experimental plots.

Tabla 2. Manejo de las parcelas experimentales.

	First sowing	Second sowing
Soil preparation	2 harrowing operations	2 harrowing operations
Sowing date	3/21/2019	4/29/2019
Fertilization	At-sowing fertilizer 150 kg/ha of 18-46-0	At-sowing fertilizer 150 kg/ha of 18-46-0
Irrigation	8 irrigations of 30 mm= 240 mm Rainfall: 83.7 mm Total: 313.7 mm	7 irrigations of 30 mm= 210 mm Rainfall: 77.6 mm Total: 287.6 mm

Data on accumulated dry matter (kg) were analyzed through ANOVA and mean comparison (Tukey test) with Infostat, student's version (2).

Results and discussion

Sowing date and species showed no interaction (p=0.9990). March sowings were significantly more productive (p=0.0017) than April sowings. In turn, considering annual kg of dry matter/ha, oat was significantly different (p=0.0037) from rye and barley (table 3, page 35). Previous studies at INTA EEA Barnetche have also shown more productive early sowings (late February/early March), than late sowings (end of March). However, they identified barley as the most productive crop (10) while our results show oats yielding the highest dry matter. Another study conducted by INTA EEA General Villegas found no differences in dry mass accumulation among barley, triticale, oats, wheat and rye (7).

Table 3. Accumulated forage biomass (kg DM/ha/year), number of cuts, sowing to first grazing (days), availability period for grazing and days of use for oats, rye and barley during a productive cycle, 2019.

Tabla 3. Biomasa forrajera acumulada (kg MS/ha/año), número de cortes, siembra a primer pastoreo (días), período de disponibilidad para pastoreo y días de uso para avena, centeno y cebada, ciclo 2019.

	Species	Kg DM/ha year	Number of cuts	Sowing to first grazing (days)	Availability period for grazing	Days of use
First sowing 3/21/2019	Oat	7646.4 **a**	4	68	5/28 to 11/14	170
	Rye	5668.2 **b**	6	77	6/6 to 11/14	161
	Barley	5682.1 **b**	4	57	5/17 to 10/29	165
First sowing average		**6332.2 A**				**165**
Second sowing 4/29/2019	Oat	6217.5 **a**	2	155	1/10 al 29/10	28
	Rye	3524.5 **b**	4	127	3/9 al 14/11	41
	Barley	4206.5 **b**	2	150	26/9 al 22/10	26
Second sowing average		4649.5 **B**				**32**

Means in capital letters indicate significant differences among sowing dates ($p<0.05$).

Means in lowercase letters indicate significant differences among species ($p<0.05$).

Medias en mayúsculas indican diferencias significativas entre fechas de siembra ($p<0,05$).

Medias en minúsculas indican diferencias significativas entre especies ($p<0,05$).

The number of cuts obtained (table 3) in each species differed from other growing sites in Argentina. Experiences at INTA Manfredi report 4 to 5 cuts for oat cultivars and 3 cuts for rye cultivars (14). In this sense, local studies are especially relevant since environment and crop management might condition productive performance. Special emphasis lies on cutting time since such a decision may affect yield. In this study, cutting was made at 40 cm plant height, while in Manfredi, cuts were made at 25-30 cm plant height.

On the other hand, the number of days from sowing to grazing was lower in March than in April (table 3), meaning that for earlier sowings, the first grazing can occur earlier, as observed in Alto Valle de Río Negro (3) and Bordenave, Province of Buenos Aires (15).

Concerning forage availability period for grazing purposes, sowing in March provides forage between May and October/November, depending on the species, while if sowing occurs during the second half of April, the first grazing can occur in early September. Considering forage availability periods of early sowed crops, they may constitute an alternative to dormant alfalfa, from late April/early May to late October/early November.

Table 4 (page 36) shows cutting dates and yield for each treatment. For the March sowing date, barley was the most precocious forage, first cut on May 17, agreeing with the results obtained in Bolívar, Buenos Aires, where barley also resulted to be the most precocious species (10). By contrast, during April sowing, the most precocious forage was rye, first cut on September 3. On both sowing dates, rye production remained steady until mid-November. It should be pointed out that the cutting criteria adopted in this paper, only considering forage height, may have influenced the results obtained since such height could be related to different phonological stages of forage crops.

Table 4. Cutting dates and dry matter obtained from each forage crop, 2019.
Tabla 4. Fechas de corte y materia seca obtenida de cada verdeo invernal, 2019.

		First sowing 3/21			Second sowing 4/29		
		Oats	**Rye**	**Barley**	Oats	**Rye**	**Barley**
Cut 1	**Date**	**28-may**	**6-jun**	**17-may**	**1-oct**	**3-sept**	**26-sept**
	kg DM/ha	1494.2	1128.1	1568.1	4974.2	1728.2	3429.2
Cut 2	Date	26-sept	1-ago	12-sept	29-oct	1-oct	22-oct
	kg DM/ha	4903.9	863.33	2871.68	1243.4	968.0	777.27
Cut 3	Date	22-oct	3-sept	10-oct		22-oct	
	kg DM/ha	792.2	2589.6	1032.8		475.9	
Cut 4	Date	14-nov	26-sept	29-oct		14-nov	
	kg DM/ha	456.2	422.98	209.47		352.4	
Cut 5	Date		22-oct				
	kg DM/ha		356.23				
Cut 6	Date		14-nov				
	kg DM/ha		307.97				

Conclusions

Early sowings (March) yielded more forage than late sowings (April) in all three species. Forage availability period for grazing purposes, as well as cutting number, was higher for March sowings.

Considering environmental effects and the adopted cutting criterion, oats yielded more forage during both sowing dates.

The obtained results show that winter forage crops could constitute a forage resource for livestock systems in Mendoza, which could be complemented with alfalfa.

Future studies should compare cultivars and cutting criteria, and include forage quality determinations.

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Comparison of visual risk assessment methods applied in street trees of Montevideo city, Uruguay

Comparación de métodos de evaluación visual del riesgo aplicados en árboles de veredas de la ciudad de Montevideo, Uruguay

Cecilia Ameneiros [1], Paulina Fratti [1], Agustina Sergio [1], Ana Paula Coelho-Duarte [1*], Mauricio Ponce-Donoso [2], Óscar Vallejos-Barra [2]

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Abstract

Risk assessment of urban trees is an incipient practice in Latin America, generally performed with foreign methods, due to the lack of qualified personnel and locally validated or adapted methodology. This article evaluates the application of three methods on street trees in Montevideo city, Uruguay: Tree Hazard Risk Evaluation and Treatment System (THREATS), Quantified Tree Risk Assessment (QTRA) and Best Management Practices - Tree Risk Assessment (ISA BMP). Three assessors with similar experience applied three methods in 36 trees of three widely used genera, totaling 324 assessments and 1,296 data. The methods were decomposed into the components: Likelihood of Failure, Likelihood of Impact, Consequence and Risk Rating. The data were statistically analyzed through a generalized linear mixed model ($p<0.05$), for the factors: assessor, method, genus, and their interactions. Results showed no significant differences among assessors, but there were differences among methods, specifically for the Likelihood of Impact and Risk Rating components. The ISA BMP method presented higher means in these last two components. Still, this method is suggested for street trees in Montevideo until a more appropriate method is adapted or developed for local conditions.

Keywords
arboriculture • hazard tree • risk component • tree risk • urban forest

1 Universidad de la República . Facultad de Agronomía. Departamento Forestal. Av. Garzón 78012900. Montevideo. Uruguay. * paula.coelho@fagro.edu.uy
2 Universidad de Talca. Av. Lircay s/n. 3460000. Talca. Chile.

Resumen

La evaluación del riesgo de árboles urbanos es una práctica incipiente en América Latina, debido a la falta de personal calificado y métodos locales validados o adaptados, debiendo utilizarse métodos foráneos. Este artículo evalúa la aplicación de tres de estos métodos en árboles de veredas de la ciudad de Montevideo, Uruguay: Tree Hazard Risk Evaluation and Treatment System (THREATS), Quantified Tree Risk Assessment (QTRA) and Best Management Practices - Tree Risk Assessment (ISA BMP). Estos fueron aplicados por tres evaluadores con similar nivel de experiencia, en 36 árboles de tres géneros ampliamente utilizados, totalizando 324 evaluaciones y 1.296 datos. Los métodos fueron descompuestos en los componentes: Probabilidad de Falla, Probabilidad de Impacto, Consecuencia y Clasificación del Riesgo. Los datos fueron analizados estadísticamente a través de un modelo lineal generalizado mixto ($p<0{,}05$), considerando los factores: evaluador, método, género, y sus interacciones. Los resultados no muestran diferencias significativas entre evaluadores, pero sí entre métodos, específicamente para Probabilidad de Impacto y Clasificación del Riesgo. El método ISA BMP presentó mayores promedios en estos dos últimos componentes, aun así, se sugiere su uso para árboles ubicados en calles de Montevideo mientras no se desarrolle o adapte un método a las condiciones locales.

Palabras clave

arboricultura • árbol peligroso • componentes del riesgo • riesgo del árbol • bosque urbano

INTRODUCTION

Urban trees (UT) take part in the physiognomic and structural configuration of cities (2), as fundamental elements of well-being in urban landscape and environment (10, 24). Given the importance of UT, keeping them in the best possible conditions turns relevant. This implies incorporating risk management (32), favoring people, goods, and activities at the same time.

Tree development in a constantly changing environment presents new challenges, especially related to management. As climate change progresses, trees live less than expected (29) causing damage to infrastructure, requiring extra maintenance, and exposing the community to higher risks, resulting in additional management costs (2). In this sense, good UT management should minimize costs and maximize benefits (21).

Although eliminating risks turns impossible, controlling tree damages (2, 6) allows reaching an acceptable risk level for stakeholders (22). Trees exceeding this level are considered dangerous (2).

Initiatives developing visual assessment methods for UT risk date from 1990 (6, 7, 9, 20, 23). These methods can be classified into qualitative, quantitative, or semiquantitative methods, depending on the structure used for categorization of each risk component (13). For a method to be incorporated as a management tool it must be complete, credible, feasible (substantiated), reliable, repeatable, robust, simple, and valid (22). In general, different methods are organized according to the components "likelihood of failure" and "likelihood of impact", with possible "consequences" of the eventual failure for people or property (31), as well as a corresponding "risk rating". This decomposition in components is a useful way to analyze method applicability (3, 17).

UT likelihood of failure is related to tree defects, with the most likely-to-fail trait being the most relevant when assessing a potential failure directly related to the potential consequences (3). The likelihood of impact is associated with the area that the failed part or entire tree can impact -the target zone-, related to the occupancy rate of people, goods and services potentially impacted (20). Therefore, high-use public spaces require the best attention (7, 15). Tree health and condition (7), as well as the targets, failing part size, falling distance and target zone (6, 7, 31), influence consequence (i.e. damage caused by the part of the tree that affects the targets, 19).

As mentioned, risk rating is calculated in quantitative, qualitative, or semiquantitative terms (6, 13, 20). In quantitative terms, real values are estimated for consequences and likelihoods. For qualitative assessment, expressions such as “low”, “moderate”, and “high” are used. Finally, semiquantitative risks may be a sum or multiplication of the components, associated with a scale, whether linear, logarithmic, or other. Nonetheless, finding methodologies that use different scales for different components, is possible (9).

Regardless of the method used, risk assessment should reduce uncertainty and help manage the risk. This should be of assistance in deciding whether to adhere to an existing method or adapt a previous one, particularly in the case of those countries that do not possess their own (26). Therefore, method evaluation tests different factors, assessors, trees, and sites, ensuring adequate reliability and repeatability (17, 18, 19, 22, 26).

Studies show significant differences when comparing risk ratings of different visual assessment methods (3, 22, 26) and assessor performance (3, 19, 22). Furthermore, when analyzing each component, some authors (19) observe greater variability in likelihood of impact than in likelihood of failure.

Considering that the methods available were developed in Anglo-Saxon countries, in other countries, especially in Latin America (1), method adaptation or development is still incipient, with few available studies (3, 4, 14, 26).

The aim of this article is to compare three methods of UT visual risk assessment qualifying their performance and possible adaptation to assess UT in Montevideo city.

Materials and methods

Fieldwork was carried out in December 2018, by assessing streets in different neighborhoods of Montevideo (34°54’04.3” S, 56°08’18.4” W and 136 m a. s. l.), Uruguay. Average temperatures range from 11°C in winter to 21.5°C during summer. Precipitation is spatially irregular and variable, presenting a maximum in autumn and a secondary maximum in spring (11). Northbound winds are the most frequent but less intense (< 65 km/h) while south-southeast and west-northwest winds are the most intense (> 80 km/h) (12).

In 2012, the city of Montevideo had 211,402 sidewalk trees, totaling 422 species (30). Four species within the most cultivated genera in Montevideo, were selected (30): *Melia azedarach* L., *Fraxinus excelsior* L., *Fraxinus pennsylvanica* Marshall and *Platanus* x *acerifolia* (Aiton) Willd. The decision was based on a registry of tree failures during storms in the 2012 - 2017 period, indicating that these species have failed the most. For this research, 36 trees were selected, 12 of each genus, with different scenarios of likelihood of failure, impact, and consequence, incorporating trees at all possible risk levels.

Three visual risk assessment methods were selected: “Quantified Tree Risk Assessment” (8), “Best Management Practices - Tree Risk Assessment” (6) and “Tree Hazard: Risk Evaluation and Treatment System” (9), so that all types of methodologies (quantitative, qualitative and semiquantitative) were considered (table 1, page 41). Risk ratings and the use of methods focused on street trees were also considered. These last considerations, along with assessor training, were decisive in the methodological selections.

The assessment methods used provide different final risk ratings, and evaluate each component, resulting in different qualitative scales or quantitative evaluations. For that reason, data analysis was standardized according to Coelho-Duarte *et al.* (2021).

Due to the limited availability of trained personnel, three assessors with basic knowledge in arboriculture applied the methods. These people received prior training consisting of theoretical and practical capacitation, totalizing about fourteen hours.

Each tree was measured considering height (m), diameter at breast height (DBH) (m) and crown projection diameter in N-S and E-W directions (m).

Three hundred and twenty-four assessments were analyzed. ANOVA considered the methods, genera, assessors, and the interaction between them as sources of variation. For the ANOVA, a generalized linear mixed model ($p < 0.05$) selected “tree” effect as random factor, since the three methods were applied in the same trees. When significant differences were found, means were compared using Fisher’s Least Significant Difference (LSD) test ($\alpha = 0.05$). The data were analyzed with the glmer function of R’s lme4 library (25), interconnected to InfoStat software version 2020 (5). Plots were developed using SigmaPlot software version 12 (Systat Software Inc.).

Table 1. Method characteristics.
Tabla 1. Características de los métodos.

Method	Characteristics
THREATS Tree Hazard: Risk Evaluation and Treatment System (9)	- Semiquantitative method. - Developed in the U.K. - After assessing tree risk, it provides recommendations for risk mitigation. - Free access and available online.
QTRA Quantified Tree Risk Assessment (8)	- Quantitative method. - Developed in the U.K. - Assumes that high risks would only occur in areas with high occupancy rate of targets or with high value properties. In case of low occupancy rate or low value, properties assessing the structural weakness of the tree would not be necessary. - The ranking is divided into four ranges defining a risk threshold under 1:10.000. - The method is available online free but requires training and licensing. - The management risk is based on a cost/benefit ratio (ALARP).
ISA BMP Best Management Practices (6)	- Qualitative method. - Developed in the USA. - Uses decision matrix for risk rating. - Includes an evaluation of biological and mechanical variables. - The method has an extensive and detailed form including a residual risk for each treatment recommended for the tree. - It can recommend the use of diagnostic equipment for advanced assessment. - Training is necessary for certification.

Results

The studied trees had different crown sizes given by reduction pruning intended to adequate size to the available space. Specimens of *Platanus* were mostly located in avenues with wide sidewalks and were considerably larger in height and crown diameter than *Melia* trees, which were more abundant in streets with narrower sidewalks. *Fraxinus* individuals were of smaller size, with lower DBH than the other genera (table 2).

Table 2. Mean dendrometric values per genus.
Tabla 2. Valores promedios de las medidas dendrométricas por género.

	Fraxinus	*Melia*	*Platanus*
Height (m)	10.8 ± 2.3	15.6 ± 4.2	21.6 ± 3.9
DBH* (m)	0.4 ± 0.1	0.7 ± 0.2	0.7 ± 0.2
CD N-S* (m)	8.0 ± 2.6	9.9 ± 2.3	13.5 ± 3.4
CD E-W* (m)	8.19 ± 1.55	10.2 ± 2.2	13.7 ± 3.7

*DBH = Diameter at Breast Height; CD N-S = Crown Diameter N-S; CD E-W: Crown Diameter E-W.

*DBH = Diámetro a la Altura del Pecho; CD N-S = Diámetro de Copa N-S; CD E-W: Diámetro de Copa E-O.

Regarding risk assessments, no interactions were found between factors. The results showed significant differences among methods only for likelihood of impact ($p = 0.016$) and risk rating ($p = 0.046$) (figure 1b and figure 1d, page 42). Among genera, differences were only found for likelihood of impact ($p = 0.013$) (figure 2, page 42), while no significant differences were found among assessors for any of the components.

Black points represent the adjusted mean; stars depict outliers; white circles show medians. Dissimilar letters denote statistically significant differences in mean ratings for LSD Fisher test (α = 5%).

Puntos negros representan la media ajustada; estrellas negras son valores atípicos; letras diferentes denotan diferencias estadísticamente diferentes en las medias determinadas con una prueba de LSD Fisher (α = 5%).

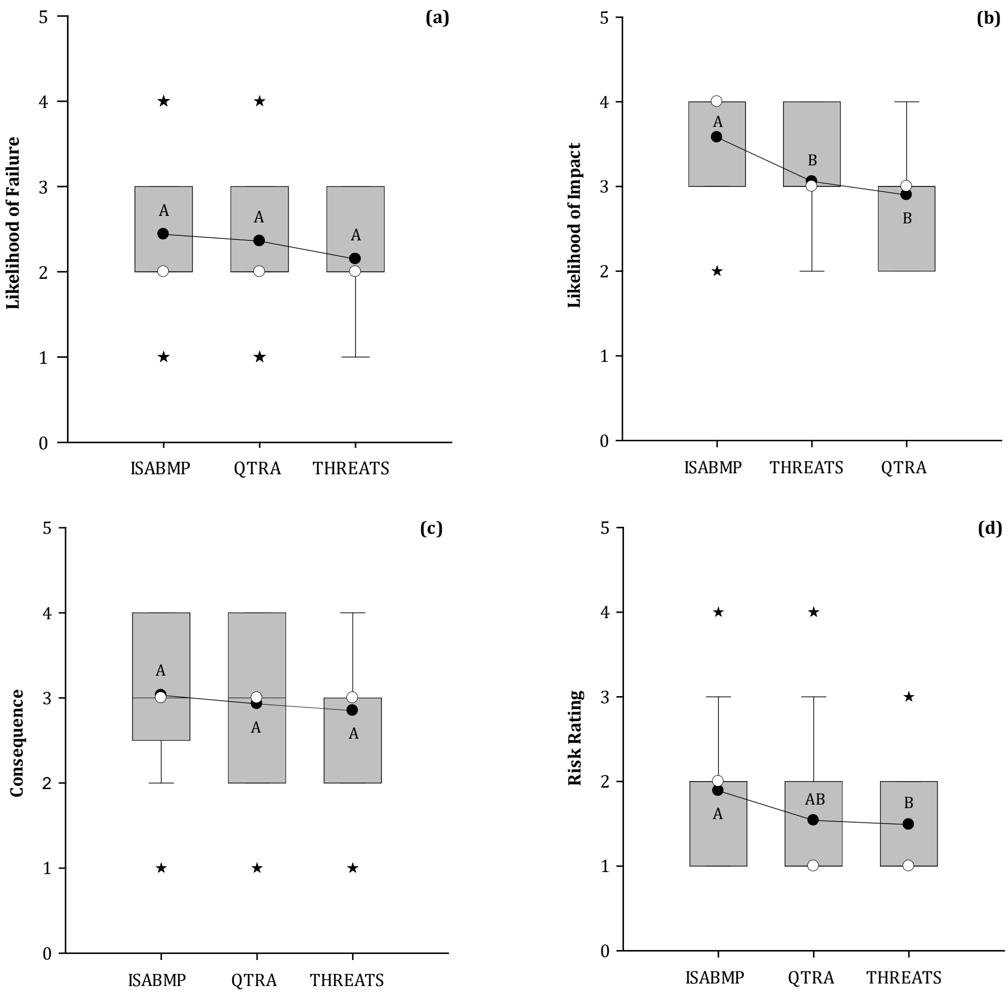

Figure 1. Boxplot (bars): (a) likelihood of failure, (b) likelihood of impact, (c) consequence, and (d) risk ratings for the three methods of visual assessment.

Figura 1. Diagrama de caja (barras): (a) probabilidad de falla, (b) probabilidad de impacto, (c) consecuencia, (d) clasificación del riesgo para los tres métodos de evaluación visual.

Black points represent adjusted mean; stars depict outliers; white circles are medians. Dissimilar letters denote statistically significant differences in mean ratings as determined with LSD Fisher test (α = 5%).

Puntos negros representan la media ajustada; estrellas negras son valores atípicos; letras diferentes denotan diferencias estadísticamente diferentes en las medias determinadas con una prueba de LSD Fisher (α = 5%).

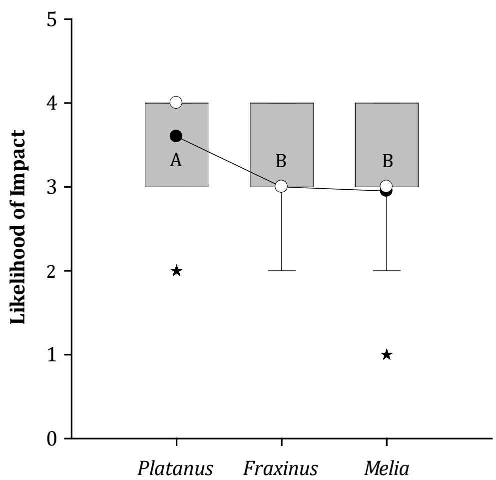

Figure 2. Boxplot (bars): likelihood of impact by genus.

Figura 2. Diagrama de cajas (barras): probabilidad de impacto por género.

Results per component

Likelihood of failure

No significant differences were found for any factor. Results distribution showed 94.4% (QTRA), 93.5% (ISA BMP) and 85.2% (THREATS) in the standardized indices 2 and 3 (figure 3). Essentially, likelihood of failure of the evaluated individuals was possible/probable. It must be noted that for the THREATS method, 14.8% of the assessments are located in the standardized index 1, referring to defect absence or minor defect presence, and none in index 4, which represents an imminent failure.

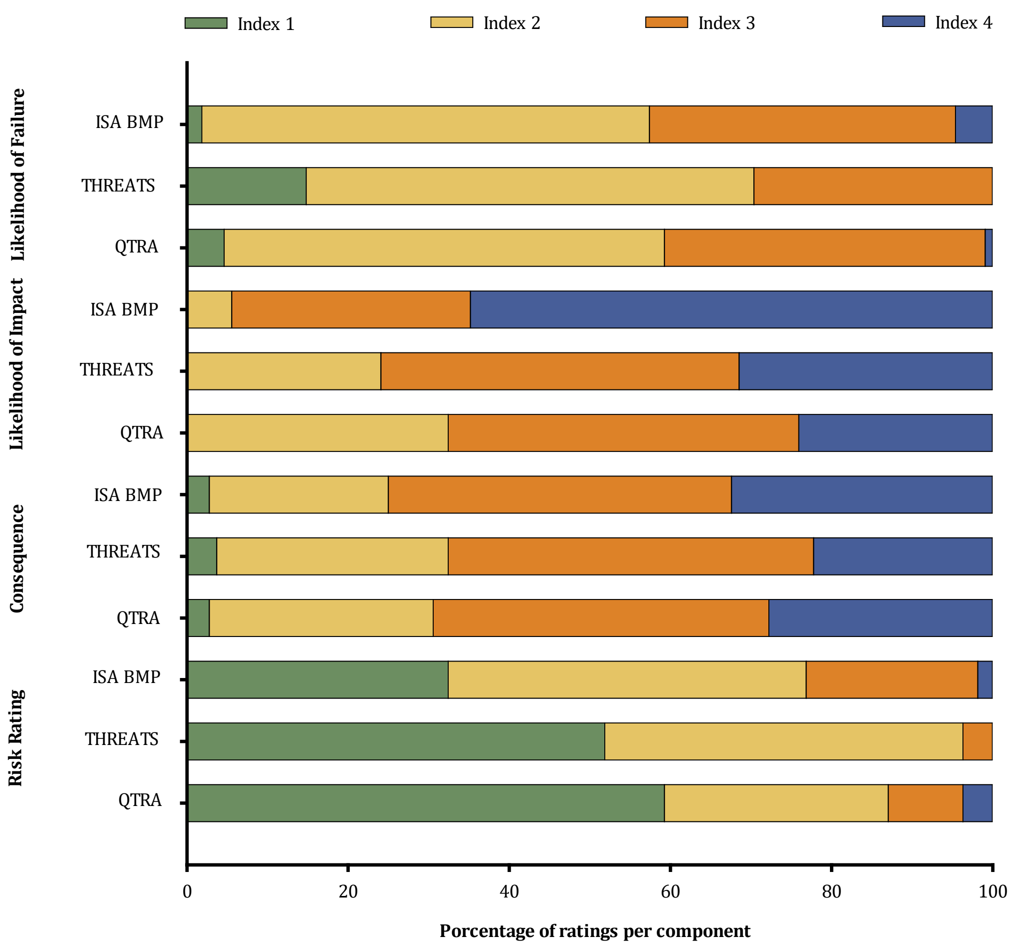

Figure 3. Assessment distribution by component for three methods (ISA BMP, THREATS and QTRA).

Figura 3. Distribución de las evaluaciones por componente para tres métodos (ISA BMP, THREATS y QTRA).

Likelihood of impact

The methods resulted in two homogeneous groups for this component (figure 1b, page 42), with the ISA BMP method bringing about the highest mean. None of the methods resulted in the standardized index 1. For QTRA and THREATS the distribution was similar within the standardized indices 2, 3 and 4, while in ISA BMP 64.8% of the results were in the standardized index 4 (figure 3). For both QTRA and THREATS it was possible to discriminate the highest occupancy rates effectively (figure 4, page 44).

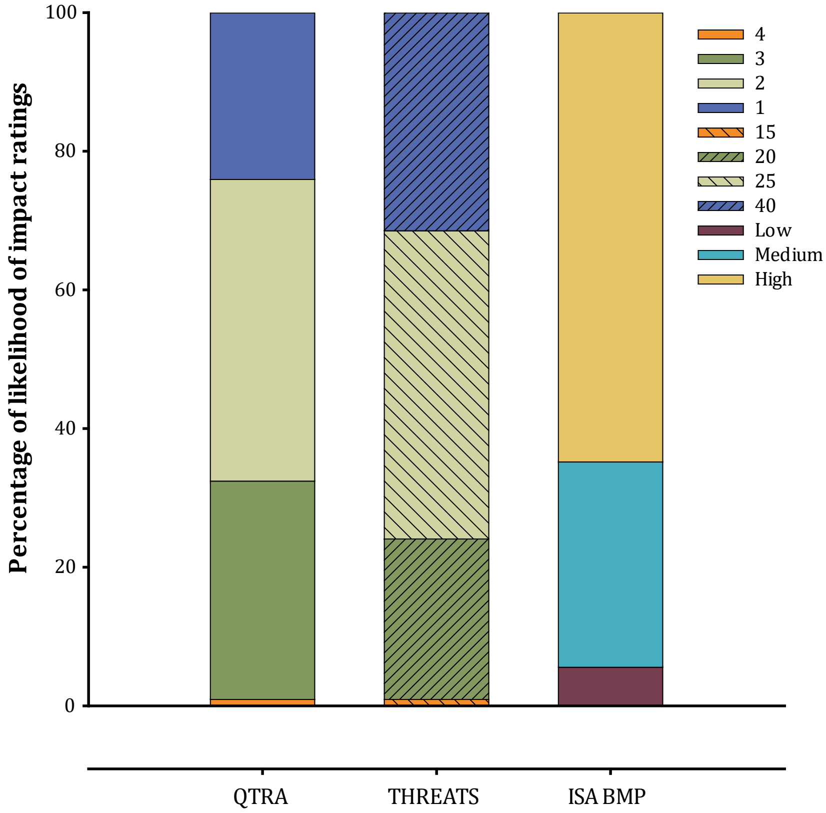

Figure 4. Assessment distribution using original ranges in each method (QTRA: 1, 2, 3, 4; THREATS: 15, 20, 25, 40; ISA BMP: Low, Medium, High) for likelihood of impact.

Figura 4. Distribución de las evaluaciones utilizando los rangos originales de cada método (QTRA: 1, 2, 3, 4; THREATS: 15, 20, 25, 40; ISA BMP: Low, Medium, High) para la probabilidad de impacto.

Consequence

No significant differences were found among factors. Thus, for the "most likely-to-fail part", branches represented 77%, 95% and 74% in *Fraxinus*, *Platanus* and *Melia* respectively; while 23%, 24% and 5% resulted for the trunk. As for the entire tree, only Melia had a 2% of the total assessments. Concerning branches, valuations fluctuated, between 11 and 16 cm.

Risk rating

The methods yielded two groups, with significant differences between ISA BMP and THREATS (figure 1d, page 42). ISA BMP resulted in "moderate" risk, even when the other components had the highest values amongst these results, No ratings were found in the "extreme" category for the THREATS method.

Discussion

Other studies had found similar results when considering the same methods (3), with the addition of significant differences for likelihood of failure. Significant differences among the genera for the likelihood of impact (figure 2, page 42) could be explained by tree location since *Platanus* sp. were located on avenues where vehicle and pedestrian circulation is constant, while *Fraxinus* sp. and *Melia* sp. were located in low-traffic streets.

The lack of significant differences among assessors for any of the components differs from previous results (3, 19). However, further perception studies state that individuals of equal age (16), gender, educational level (16, 28), and social ties (27) tend to judge possible risks in a similar way, explaining our results.

Discussion per component

Likelihood of failure

The THREATS method presented a different dispersion, in accordance with Coelho-Duarte *et al*. (2021). Assessor equal training level at the moment of categorizing likelihood of failure could explain the observed non-significant differences. In this regard, other authors (19) founding differences among assessors for this likelihood, highlighted the component as presenting the lowest variability among them. A different research distinguished knowledge levels among assessors, finding differences in likelihood of failure between a more experienced group and a less experienced one (3).

Likelihood of impact

The lack of results for the standardized index 1 for all methods, could be explained by the "rare occupancy" rate of street trees. The distinct predomination of index 4 for ISA BMP may be due, on the one hand, to the fact that the ISA BMP proposes four categories to assess the impact, while the other tested methods propose six, resulting to be more similar. On the other hand, the difference could be also due to the standardization, which might allow the other two methods a better discrimination between the highest categories. Therefore, the qualitative assessment of the ISA BMP method could be overestimating this component, as previously found (3).

For a more precise measurement of the occupancy rate, traffic counters have been proposed (15), reducing variability among methods, after reducing assessor subjectivity.

Consequence

As each method has a particular way of consequence evaluation in terms of tree-part size and the attributes to be considered, significant differences were expected among them. No difference, as already observed (3), may be associated with the branches being the part with the highest likelihood of failure in most of the assessments.

No significant differences among assessors in consequence analysis differ from that previously reported (19), where the second component showed the highest variability among assessors.

Risk rating

When observing the ISA BMP matrix and the obtained risk rating (6), most of the possible combinations between components resulted in "low" risk level.This explained that the final average resulted lower than individual risk components.

Low ratings in the "extreme" category for THREATS (index 4, figure 3, page 43) could be influenced by the likelihood of failure component, as indicated by Coelho-Duarte *et al.* (2021).

Regarding the observed assessment dispersion (figure 1d, page 42), the ISA BMP and QTRA methods resulted in the more adequate tree risk classifications, with a reduced number of trees at the "extreme" level, similarly to that previously found (3). The difference between both methods is that QTRA resulted in 59.2% of the assessments at "low" level (figure 3, page 43), in which the trees would not need treatment, while ISA BMP yielded 44.4% "moderate" level, where treatment depends on the benefits outweighing handling costs (8).

Unlike that reported in other studies (3, 19, 22) in our study, risk assessors were not significantly different from each other. In this context, the used basic visual assessment methods proved to efficiently determine tree fall risks, complying with that already proposed (22). However, in the case of Montevideo, not all methods resulted completely appropriate. Some of the descriptors used do not apply to meteorological conditions and city infrastructure, such as urban furniture, pedestrian and vehicular transport, and space for the tree itself, amongst others.

Considering the abovementioned, we observed that the ISA BMP method resulted in the best option, with defect analysis in depth and residual risk designation. When recommending management, the THREATS method is the only presenting a list of treatments, stipulating a period for their performance and re-inspection. It is stated that the qualitative features of this method use some ambiguous descriptors (22), thus, any suggested treatment could not be necessary when ambiguously interpreting them. For its part, the QTRA method provided few details during the assessment, probably making subsequent risk management more difficult, but considering consequent risk costs (8).

The lack of certified arborists for tree risk assessment is evident in many cities of the Latin American Region. In this case, the number of assessors is compensated by the amount of data analyzed, reason for considering the methodology as adequate for the exploratory and descriptive characteristics of this research. Therefore, we recommend increasing the number of assessors and trees in future research.

Conclusions

No significant differences were found among assessors, allowing the application of these methods by those with similar training level. Additionally, this would constitute encouraging standard trainings for all assessors.

Methods for the likelihood of impact and risk rating showed significant differences. In both cases, the ISA BMP method presented the highest results, being the most relevant comparison aspects.

Differences among genera were found for the likelihood of impact component, influenced by target occupation rate and characteristics.

Compared to the ISA BMP The QTRA and THREATS methods, in the highest categories of likelihood of impact, distinguished two ranges.

The absence of descriptors and categorizations, and application time resulted characteristics to be improved, where ISA BMP exceeded the limit for application time. Still, ISA BMP method is suggested for street trees risk assessment in Montevideo, until an appropriate method including treatment recommendations and guidelines for risk management is adapted or developed.

Regardless of the method used, we suggest complementing visual assessment with advanced equipment for those trees classified as higher risks.

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Effect of bioslurries on tomato *Solanum lycopersicum* L and lettuce *Lactuca sativa* development

Efecto de bioles en el desarrollo de tomate *Solanum lycopersicum* L y lechuga *Lactuca sativa*

Ivan Funes-Pinter [1, 2, 3 *], María Victoria Salomón [1, 2], José Nicolás Martín [1, 2],
Ernesto Martín Uliarte [3], Andrea Hidalgo [2]

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Abstract

Bioslurries, obtained by anaerobic digestion of fresh organic matter, are emerging as a cheaper and low-impact alternative for synthetic products in agriculture. The aim of this study was to evaluate bioslurry obtained from biogas digestion (Biog), bioslurry for plant nutrition by FAO (Bfao), and lactic fermentation (Blac) as biostimulant in tomato and lettuce plants. Based on a toxicity test, a 10% dilution was finally applied to the plants. In lettuce, Bfao and Blac significantly increased aerial biomass (2.17 ±0.54 and 2.33 ±1.13 g respectively), regarding water control (1.16 ±0.60 g), while root biomass was only increased by Bfao (1.60 ±0.44 g) compared to control (0.66 ±0.34 g). All digestates increased chlorophyll content index (CCI), while yield (Fv/Fm) and performance index (Plabs) did not show differences with water control. In tomato, only aerial biomass was significantly increased by Bfao. All digestates significantly increased CCI, while Fv/Fm was only significantly higher in Bfao and Blac, related to water control. PIabs showed no differences. In both plant species, commercial fertilizer showed significantly higher values for all parameters. In conclusion, all digestates stimulated plant growth, Bfao showed the highest effect on tomatoes and lettuce biomass followed by Blac and Biog, being a cheaper, safer and lower-impact alternative for traditional products for crop growing.

Keywords
anaerobic digestion • agroecology • organic production • plant nutrition • *Lycopersicum esculentum*

1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Av. Ruiz Leal s/n - Parque Gral. San Martín (M5500XAD). Mendoza. Argentina.
* ifunes.pinter@inta.gob.ar
2 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Almirante Brown 500 (M5528AHB). Chacras de Coria. Mendoza. Argentina.
3 Agrotecnología Sostenible. Estación Experimental Mendoza. Instituto Nacional de Tecnología Agropecuaria (INTA EEA Mendoza). San Martin 3853 (M5507EVY). Chacras de Coria. Mendoza. Argentina.

Resumen

Los bioles son bioinsumos obtenidos de la digestión anaeróbica de materia orgánica, que surgen como una alternativa económica y de bajo impacto para la agricultura. El objetivo del presente trabajo fue evaluar los bioles obtenidos de: la producción de biogás (Biog), elaborado para la nutrición vegetal con base en la FAO (Bfao), y de una fermentación láctica (Blac), para utilizarse como bioestimulantes en lechuga y tomate. Sobre la base del análisis de toxicidad, la dilución al 10% fue seleccionada para aplicarse en las plantas. En lechuga, Bfao y Blac incrementaron significativamente la biomasa aérea (2,17 ±0,54 y 2,33 ±1,13 g respectivamente), referente al control (1,16 ±0,60 g), mientras que la biomasa radical presentó mayores valores solo en Bfao (1,60 ±0,44 g), comparado al control (0,66 ±0,34 g). Los bioles incrementaron el contenido de clorofila (CCI), mientras que los índices de rendimiento (Fv/Fm) y de desempeño (Plabs) no se diferenciaron del control. En tomate, solo la biomasa aérea fue significativamente mayor en Bfao, todos los bioinsumos incrementaron significativamente CCI, mientras que Fv/Fm solo por Bfao y Blac, y PIabs no mostró diferencias respecto al control. En ambos, lechuga y tomate el fertilizante comercial fue el tratamiento con mayores valores, sin embargo, todos los bioinsumos estimularon el crecimiento vegetal. El mayor efecto fue producido por Bfao, seguido de Blac y Biog, siendo una alternativa adecuada para una producción vegetal económica, segura y con menor impacto.

Palabras clave

digestión anaeróbica • agroecología • producción orgánica • nutrición vegetal • *Lycopersicum esculentum*

Introduction

The Cuyo region in Argentina is an arid zone with heterogeneous and poorly structured soils with low fertility and organic matter. Thus, performing management tasks favoring higher biological activity and nutrient availability turns necessary for sustainable crop growing (1). Moreover, due to the limited information about organic amendments in these arid soils, several studies suggest an increase in crop productivity and improvement in soil properties by bio-input applications (20).

Worldwide, tomato (*Solanum lycopersicum* L) and lettuce (*Lactuca sativa*) are two important horticultural crops, being tomato the second most consumed in Argentina. These crops are mainly produced in oases located in arid zones, being Cuyo region the highest cultivated surface (35), with Mendoza province as the main tomato producer with 3,757 ha, and 265 ha for lettuce (16). However, the intensive farming of tomatoes and lettuce requires a large amount of high-cost synthetic agrochemicals to cover nutrient requirements and control diseases and plagues, thus having negative environmental impacts (5, 9).

Therefore, organic products are becoming a cheaper and low-impact alternative to synthetic ones. In this sense, biopesticides and biofertilizers developed locally represent a viable alternative for sustainable crop management, lowering environmental negative effects and production costs. Bioproducts are biologically active supplies that promote plant growth by different strategies (10), such as increasing nutrient availability, producing phytohormones and antibiotics, and/or competing against pathogens (26, 27). Furthermore, natural compounds may quickly biodegrade, due to microbiologic coevolution and decomposition metabolic pathways (7).

Bioslurry or digestate, obtained by anaerobic digestions of fresh organic matter, presents adequate characteristics for plant growth. Most of the available bibliography refers to bioslurry as the byproduct or residue of biogas production with biofertilizer and biocontroller properties against plant diseases (8, 17). The liquid fraction derived from anaerobic digestions can retain nutrients and microorganisms. It has also been tested as biofertilizer, soil amendment, and even for bioremediation, with promising results (29, 30, 36).

By-products of anaerobic digestions have been also developed as plant biostimulants, with no biogas generation. The Food and Agriculture Organization (FAO), proposed a protocol to obtain a bioslurry specifically designed for plant nutrition and growth promotion, based on elemental and microbial content in the final product (12). Other authors have reported lactic fermentation for obtaining adequate biostimulants for plant growth, showing promising results (22). Nevertheless, there is a lack of information about the properties of these non-methanogenic bioslurries in plant nutrition and antipathogen effectiveness.

The study evaluated three different bioslurries obtained from different raw materials and elaboration processes, as biofertilizers for tomato and lettuce. The hypothesis stated that anaerobic digestions generate by-products rich in nutrients and microorganisms that increase plant development.

Methods

Digestates elaboration

Three different digestates or bioslurries were tested as plant biostimulants: bioslurry obtained from biogas digestion (Biog); bioslurry designed for plant nutrition by FAO (Bfao); and a lactic fermentation lixiviate (Blac). Biog was obtained from biogas digestion of a traditional mixture of water:goat manure in a 9:1 ratio. The biodigester was built with two 200 μm polyethylene layers in a 1.25 m^3 tubular design intended to be semi-buried in cold climates, for integrated solar gain systems and insulated enclosure. During Biog production, the biodigester was operated at 25 °C with a hydraulic retention time of 60 days and fed twice a week with a 7 kg load of goat manure and 60 kg of water. Bfao was brewed according to FAO protocol (2013): An anaerobic process was carried out in a 200 L PET recipient, with a screw cap and a gas trap containing 10 kg of fresh vegetal material, 60 L of fresh goat manure, 3 kg of wood ashes, 4 kg of bentonite, 500 g of chicken eggshells, 3 kg of bone ashes, 5 L of cow milk, and free chloride water to a final volume of 170 L. After 3 months of storage, the product was filtered and stored in darkness at 15-20 °C. Finally, Blac elaboration protocol was based on Quirós *et al.* (2004): a mixture of 1 L of commercial rice and 2 L of chlorine-free water was left to settle for 48 h. Later, the lixiviate obtained was supplemented with 6 L of milk and stored at 30 °C for 3 days. Finally, it was filtered and stored until use.

Bioslurries characterization

Toxicity test

Lactuca sativa var Grandrapids seeds were used for toxicology tests according to US EPA (1996). Seeds were previously tested for germination power and seed viability using sterile distilled water. Then, seeds were superficially sterilized with 70% ethanol and exposed to the biostimulants as follows: 20 seeds were placed on a filter paper in 90 mm Petri dishes, exposed to 0, 10, 25, 50, and 100% dilutions of each product by triplicate, and maintained at 20 °C in darkness for 5 days. Control treatment and dilutions were performed with well water (H_2O), also used for product brewing. To evaluate the toxic effect, total germination, and hypocotyl/root elongation, were analyzed.

Physico-chemical analyses

To perform a basic characterization, pH, CE, and macronutrients were analyzed in each bioproduct by duplicate. Total nitrogen (N) was determined in dry samples by Kjeldahl and steam trawl distillation method (4). Phosphorus (P) was colorimetrically analyzed by HCl extraction with ammonium methavanadate, ammonium molybdate, and nitric acid solution. Absorbance at 420 nm was measured with a UV-VIS Milton Roy spectrophotometer. Finally, K was determined by flame atomic absorption spectrophotometry (32).

Pot assays

To evaluate bioslurries effect on plant growth, seeds of *Lactuca sativa* var Grand rapids and *Solanum lycopersicum* var Platense were germinated and grown in seedling trays for 15 days, with basal fertilization of 500 mg of commercial fertilizer (KSC® 2 NPK 23-5-5, Timac Agro USA). Seedlings were transplanted into a 0.5 L pot containing perlite:peat (1:1) and grown under greenhouse conditions (23±2 °C, 30% humidity, and natural 16/8 h photoperiod due summer season). All plants were irrigated with well water every 48 h to maintain field capacity during the assay. After transplant, homogeneous 10 cm plants were selected for treatment initiation. A complete randomized block design was established with 5 treatments for each plant species (n=8): Biog, bioslurry from biogas production; Bfao, bioslurry designed for plant nutrition by FAO (2013); Blac, lixiviate of a lactic fermentation; Cont, well water; and Fert, chemical fertilization. Throughout the assay, 50 mL of each product diluted at 10% were weekly applied.

Aerial and root dry weight (DWa, DWr, respectively) were determined in 120 days old plants. Additionally, yield (Fv/Fm) as an indicator of photosystem II damage, and performance index (PIabs) as stress resistance capacity, were measured with a Chlorophyll Fluorimeter Handy Pocket PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, England). This was carried out with a leaf-clip placed on the third leaf from the apex for 20 min till dark adaptation (13).

Finally, chlorophyll content index (CCI) by absorbance was also determined in the third leaf with a chlorophyll meter (model Clorofilio, Cavadevices, Argentina).

Statistical analysis

ANOVA was performed considering the block design, and a LSD Fisher means comparison test ($p<0.05$) evaluated the effect of the digestates on seedling biomass. Shapiro-Wilk normality tests and residues regression were carried out to confirm ANOVA assumptions. Due to the lack of normality, phytotoxicity test was analyzed by non-parametric Kruskall Wallis test ($p<0.05$). InfoStat software version 2015 performed all statistical analyses (InfoStat Group, FCA, National University of Córdoba, Argentina). All data was expressed as mean ± standard deviation.

Results

Phytotoxicity test

Blac treatment decreased seed germination at concentrations of 100, 50, and 25%, while 10% did not differentiate from control even though the value was lower. Biog and Bfao did not show significant differences from control in any dilutions, except for Biog 100%, with zero germinated seeds.

Root and hypocotyl elongation was negatively affected by Blac in all concentrations, whereas Bfao and Biog 10% significantly increased these parameters. At 25%, both Bfao and Biog significantly increased plant hypocotyl while not affecting root elongation. At higher concentrations, all products showed toxicity, reducing plant elongation (table 1, page 52).

Except for Blac, no treatment differed from control at 10%, suggesting no phytotoxicity properties or plant growth stimulation. Such concentration was considered for further analyses based on these results and the bibliography. Blac 10% was included in further assays for results verification, considering that *in vitro* conclusions may be limited and different results may be expected in pot treatments.

Bioslurries characterization

Only Bfao presented an adequate pH value (Resolución 19/2019, Secretaría de Gobierno de Ambiente y Desarrollo Sustentable, Argentina; Ministry of Environment and sustainable development of Argentina). All products presented high EC levels, while Blac also presented high acidity (low pH value). Considering the phytotoxicity results, all products must be diluted, being 10% the most adequate dilution (table 2, page 52).

Effect of bioslurries on lettuce growth

There were no significant differences among biolsurries treatments. Nevertheless, Bfao and Blac showed significantly higher aerial biomass (2.17 ±0.54 and 2.33 ±1.13 g respectively), related to control (1.16 ±0.60 g; figure 1B, page 53). Root biomass was only increased by Bfao (1.60 ±0.44 g) (0.66 ±0.34 g; figure 1C, page 53). As expected, Fert was the treatment with significantly higher values of plant biomass (3.64 ±1.21 and 6.2 ±1.50 g, respectively for root and aerial dry weight, figure 1A, page 53).

Table 1. Phytotoxic effect of bioslurries on *Lactuca sativa* var Grand Rapids seeds.

Tabla 1. Efecto de fitotoxicidad de los bioles en semillas de *Lactuca sativa* var Grand Rapids.

Kruskal Wallis p= 0.05. Values are expressed as mean ±SD.

Se realizó un análisis de Kruskal Wallis con una significancia del 0,05. Valores expresados como media ±DS.

Biog: bioslurry from biogas production; Bfao: bioslurry designed for plant nutrition by FAO (2013); Blac: lixiviate of a lactic fermentation; and H_2Od: distilled water used as control.

Biog: biol proveniente de la producción de biogás; Bfao: biol diseñado para la nutrición vegetal, en base a FAO (2013); Blac: lixiviado de una fermentación láctica; y H_2Od: agua destilada usada como control.

Treatments	Seeds germinated		Root elongation (mm)		Hypocotyl elongation (mm)	
H_2Od	19.67	±0.58^{c}	19.27	±6.73^{d}	14.85	±5.68^{c}
Bfao 10%	19.67	±0.58^{c}	29.22	±7.38^{e}	36.49	±10.16^{e}
Bfao 25%	20.00	±0.00^{c}	20.67	±6.07^{d}	33.05	±8.15^{e}
Bfao 50%	18.00	±1.73^{bc}	4.5	±2.63^{ab}	11.5	±6.81^{bc}
Bfao 100%	12.67	±3.21^{abc}	1.34	±0.63^{a}	3.16	±2.42^{ab}
Blac 10%	14.33	±2.52^{abc}	1.00	±0.00^{a}	0.00	±0.00^{a}
Blac 25%	5.00	±0.00^{ab}	1.00	±0.00^{a}	0.00	±0.00^{a}
Blac 50%	0.00	±0.00^{a}	0.00	±0.00^{a}	0.00	±0.00^{ab}
Blac 100%	0.00	±0.00^{a}	0.00	±0.00^{a}	0.00	±0.00^{ab}
Biog 10%	19.00	±1.00^{bc}	26.86	±7.79^{e}	36.79	±10.71^{e}
Biog 25%	19.67	±0.58^{c}	14.05	±4.89^{c}	23.41	±8.28^{d}
Biog 50%	15.00	±1.73^{abc}	6.17	±3.12^{b}	10.00	±5.07^{bc}
Biog 100%	0.00	±0.00^{a}	0.00	±0.00^{a}	0.00	±0.00^{ab}

Table 2. Bioslurries physico-chemical characterization. Each product was analyzed by duplicate.

Tabla 2. Caracterización fisicoquímica de los bioles. Cada producto fue analizado por duplicado.

Biog: bioslurry from biogas production; Bfao: bioslurry designed for plant nutrition by FAO (2013); and Blac: lixiviate of a lactic fermentation.

Biog: biol proveniente de la producción de biogás; Bfao: biol diseñado para la nutrición vegetal, en base a FAO (2013); Blac: lixiviado de una fermentación láctica.

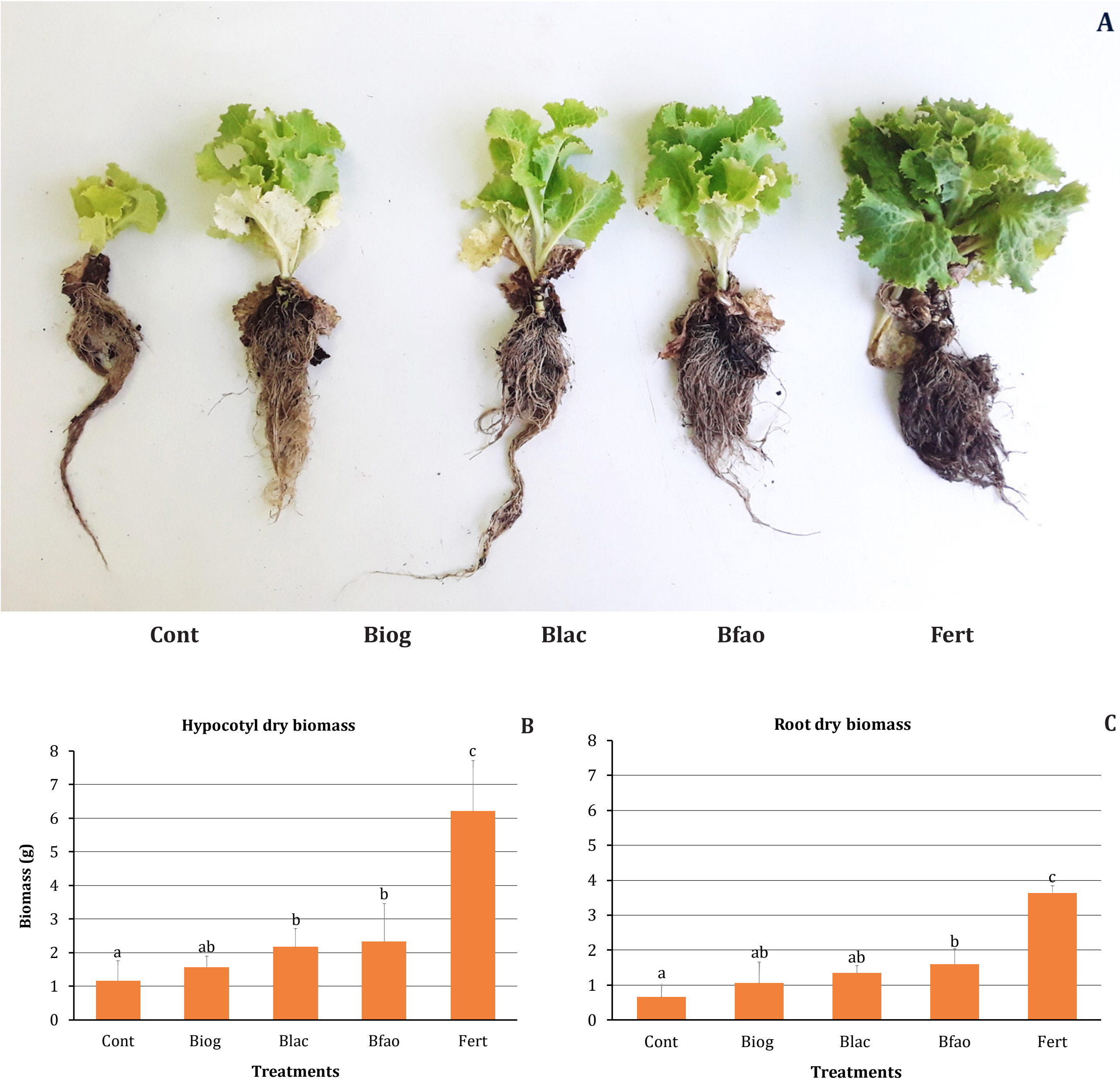
Bioslurry	pH	EC dS m^{-1}	N	P mg kg^{-1}	K
Biog	8.69	11.11	630	27	6600
Blac	3.50	8.10	1600	590	1100
Bfao	8.23	16.25	490	22	3900

CCI in lettuce plants was significantly increased by all digestates with respect to control (18.61 ±3.35), with the maximum value reached by Fert treatment (35.06 ±2.00), and followed by Bfao (30.59 ±3.70). Biog (23.33 ±1.27) and Blac (24.17 ±2.12) showed lower values and did not differentiate from each other (figure 2A, page 54).

As stress indicator, damage in photosystem II was significantly higher in Fert (0.80 ±0.03), regarding Cont (0.82 ±0.01), reaching the lowest Fv/Fm value, while Fert and Cont digestates did not differentiate (figure 2B, page 54). Oppositely, PIabs were significantly increased by Fert (4.23 ±1.85), and digestates did not differentiate from Cont (2.19 ±0.58), with Bfao showing the highest value (2.68 ±1.26, figure 2C, page 54).

Effect of bioslurries on tomato growth

Bioslurries did not significantly increase root dry biomass in tomato plants, and were significantly lower than Fert treatment. As in lettuce, Fert was the treatment with the highest root and aerial biomass (33.53 ±11.45 and 83.09 ±5.39 g for) while control showed the lowest biomass values (figure 3, page 55). Aerial biomass was significantly increased only by Bfao (10.21 ±3.05 g), in relation to Cont (5.47 ±1.38 g), while Blac and Biog did not show significant effects.



Cont: control, irrigated with water; Biog: bioslurry from biogas production; Bfao: bioslurry designed for plant nutrition by FAO (2013); Blac: lixiviate of a lactic fermentation; and Fert: inorganic fertilizer (KSC® 2 NPK 23-5-5, Timac Agro USA).

Cont: control, regado con agua; Biog: biol proveniente de la producción de biogás; Bfao: biol diseñado para la nutrición vegetal, en base a FAO (2013); Blac: lixiviado de una fermentación láctica; y Fert: suplementado con fertilizante inorgánico (KSC® 2 NPK 23-5-5, Timac Agro USA).

Values are expressed as mean ±SD. ANOVA (LSD Fischer, $p<0.05$).

Valores expresados como media ±DS. ANOVA (LSD Fischer, $p<0,05$).

Figure 1. Effect of bioslurries on *Lactuca sativa* total (A), aerial (B) and root biomass (C).

Figura 1. Efecto de los bioles en la biomasa total (A), aérea (B) y radical (Ca) de *Lactuca sativa*.

A: CCI: chlorophyll content index; B: Fv/Fm: yield index, indicators of photo system II damage; and C: PIabs: performance index, indicator stress resistance capacity. Cont: control, irrigated with water; Biog: bioslurry from biogas production; Bfao: bioslurry designed for plant nutrition by FAO (2013); Blac: lixiviate of a lactic fermentation; and Fert: inorganic fertilizer (KSC® 2 NPK 23-5-5, Timac Agro USA).

A: CCI: índice de contenido de clorofila; B: Fv/Fm: índice de rendimiento, indicador de daño del fotosistema II; y C: PIabs: índice de desempeño, indicador de la capacidad de resistir estrés. Cont: control, regado con agua; Biog: biol proveniente de la producción de biogás; Bfao: biol diseñado para la nutrición vegetal, en base a FAO (2013); Blac: lixiviado de una fermentación láctica; y Fert: suplementado con fertilizante inorgánico (KSC® 2 NPK 23-5-5, Timac Agro USA).

Values are expressed as mean ±SD. ANOVA (LSD Fischer, p<0.05).

Valores expresados como media ±DS. ANOVA (LSD Fischer, p<0,05).

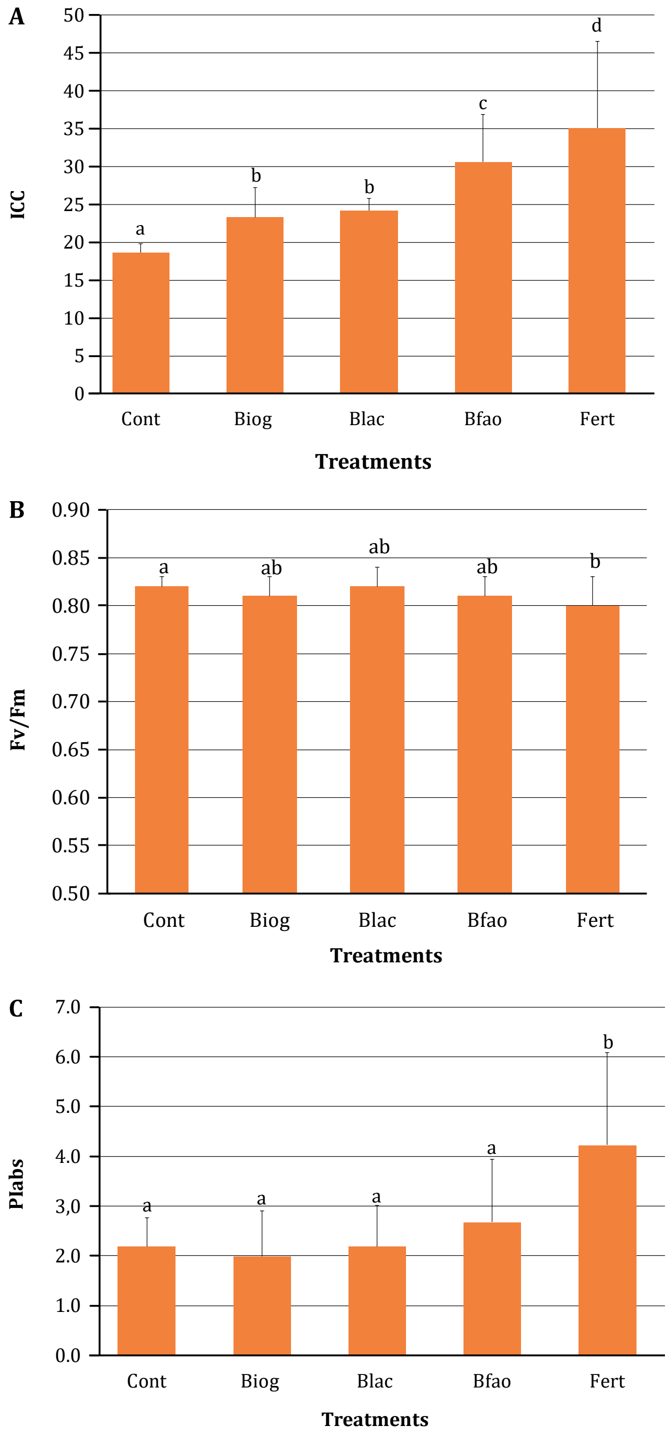


Figure 2. Effect of bioslurries on nutritional and stress indicators in *Lactuca sativa*.

Figura 2. Efecto de los bioinsumos en indicadores nutricionales y de estrés en *Lactuca sativa.*

Cont: control, irrigated with water; Biog: bioslurry from biogas production; Bfao: bioslurry designed for plant nutrition by FAO (2013); Blac: lixiviate of a lactic fermentation; and Fert: inorganic fertilizer (KSC® 2 NPK 23-5-5, Timac Agro USA).

Cont: control, regado con agua; Biog: biol proveniente de la producción de biogás; Bfao: biol diseñado para la nutrición vegetal, en base a FAO (2013); Blac: lixiviado de una fermentación láctica; Fert: suplementado con fertilizante inorgánico (KSC® 2 NPK 23-5-5, Timac Agro USA).

Values are expressed as mean ±SD. ANOVA (LSD Fischer, $p<0.05$).

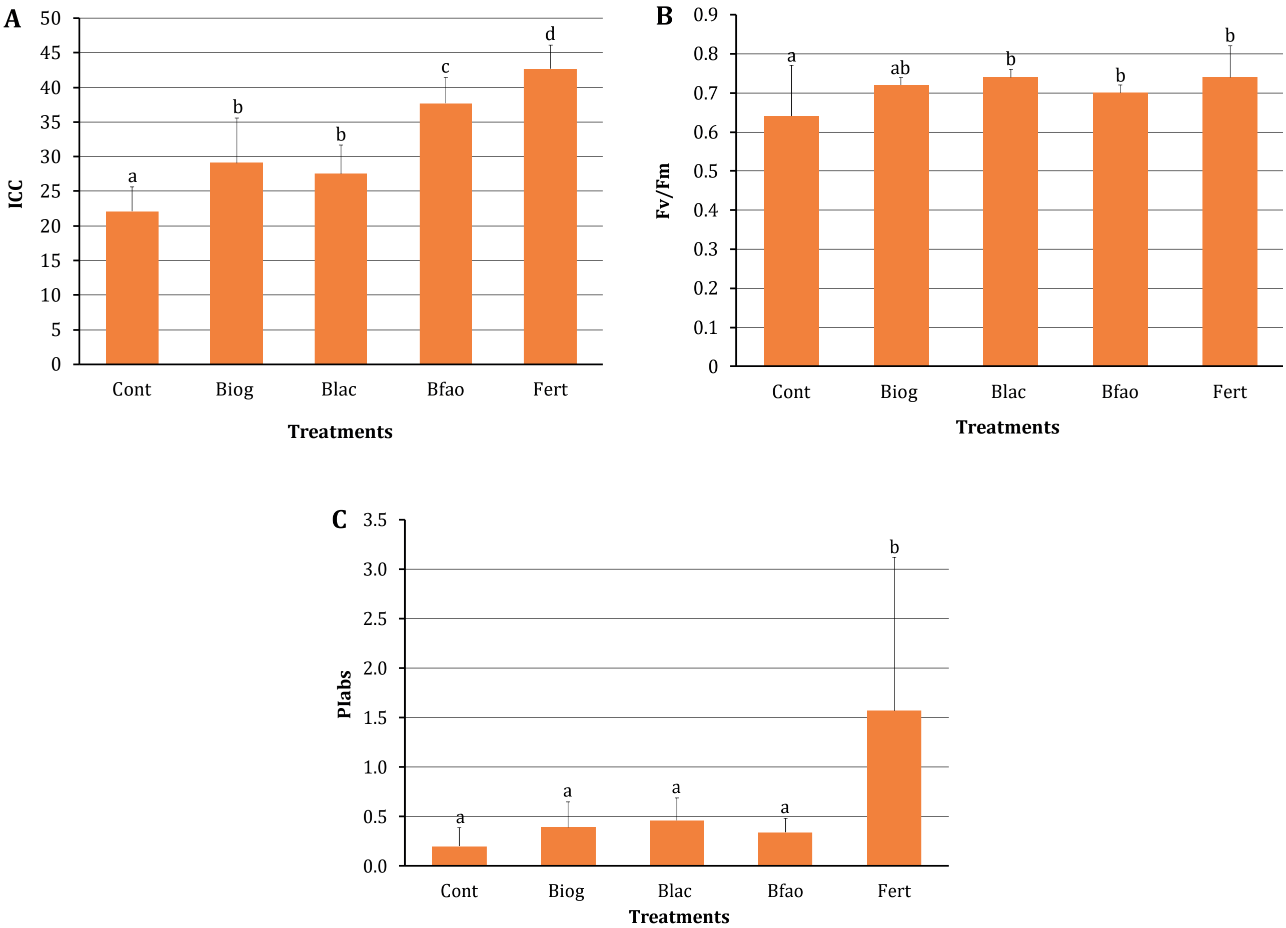
Valores expresados como media ±DS. ANOVA (LSD Fischer, $p<0,05$).

Figure 3. Effect of bioslurries on *Solanum lycopersicum* total (A), aerial (B) and root biomass (C).

Figura 3. Efecto de los bioles en la biomasa total (A) aérea (B) y radical (C) de *Solanum lycopersicum.*

All digestates significantly increased CCI in tomato plants compared to control (22.1 ±3.56). The maximum value was reached by Fert treatment (42.66 ±3.44), followed by Bfao (37.69 ±3.71). As in lettuce plants, Biog (29.04 ±6.55) and Blac (27.5 ±4.16) did not differentiate from each other and presented lower values (figure 4A).

Yield index Fv/Fm was significantly higher in Bfao (0.70 ±0.02), Blac (0.74 ±0.02) and Fert (0.74 ±0.08), with respect to Cont (0.64 ±0.13), and with no differences among them (figure 4B). PIabs was significantly increased only by Fert (1.57 ±1.55). Nevertheless, no differences were detected among digestates and Cont treatments (figure 4C).



A: CCI: chlorophyll content index; B: Fv/Fm: yield index, indicators of photosystem II damage; and C: PIabs: performance index, indicator stress resistance capacity. Cont: control, irrigated with water; Biog: bioslurry from biogas production; Bfao: bioslurry designed for plant nutrition by FAO (2013); Blac: lixiviate of a lactic fermentation; and Fert: inorganic fertilizer (KSC® 2 NPK 23-5-5, Timac Agro USA).

A: CCI: índice de contenido de clorofila; B: Fv/Fm: índice de rendimiento, indicador de daño del fotosistema II; y C: PIabs: índice de desempeño, indicador de la capacidad de resistir estrés. Cont: control, regado con agua; Biog: biol proveniente de la producción de biogás; Bfao: biol diseñado para la nutrición vegetal, en base a FAO (2013); Blac: lixiviado de una fermentación láctica; y Fert: suplementado con fertilizante inorgánico (KSC® 2 NPK 23-5-5, Timac Agro USA).

Values are expressed as mean ±SD. ANOVA (LSD Fischer, $p<0.05$).

Valores expresados como media ±DS. ANOVA (LSD Fischer, $p<0,05$).

Figure 4. Effect of bioslurries on nutritional and stress indicators in *Solanum lycopersicum*.

Figura 4. Efecto de los bioinsumos en indicadores nutricionales y de estrés en *Solanum lycopersicum*.

Discussion

In the present study, we demonstrated the ability of different bioslurries or digestates obtained from organic waste as biofertilizers, resulting in high-quality inputs for agricultural production. Generally, digestates are the result of anaerobic digestion of organic residues for energetic generation, suggesting the potentiality of anaerobic processes to reduce negative environmental impacts.

However, digestates may have high EC, exceeding the 3 dS m^{-1}, limit established for irrigation water, and potentially toxic for agriculture production (24). It may also contain high ammonia concentrations causing decreased oxygen concentration in the root system (6). According to our results, the retention time of each elaboration process seems independent to element solubilization. Bfao, with a retention time of 90 days, did not show higher NPK values than a 60 day Biog, while Blac showed the highest N and P content, with 5 days of brewing time.

In line with other studies, for plant growth, dilutions are commonly needed to reduce phytotoxic effects of pure products. In agriculture, there is no agreement among authors on the optimal dilution of digestates for maximum stimulation and minimum toxicity. Song *et al.* (2021) reported 20% as digestate optimal concentration for biostimulant usage in several horticultural crops. Nonetheless, Díaz Montoya (2017) suggested negative effects on lettuce germination at concentrations above 2-4%; and Silva *et al.* (2011) reported possible phytotoxicity at concentrations higher than 10%. Our results indicated dilutions lower than 25% for Bfao and Biog, and 10% for Blac. This suggests a high influence of the raw material used for bio-inputs brewing, determining the quality and variety of the nutrient and metabolites, more than the EC itself. Despite the higher EC of Bfao and Biog, lettuce plants showed low toxicity at a higher digestate concentration (25%), while in Blac, a 10% dilution was necessary, avoiding negative effects.

Tomato and lettuce biomass increased with the bioproducts, mainly by Bfao, due to its specific design for plant growth. Biog seemed to stimulate growth but not significantly from control, while Blac significantly increased lettuce biomass. Regardless of the high N and P content of Blac, its elaboration process is focused on lactic bacteria content and their effect on plant growth. These microorganisms serve as biofertilizers, biocontrollers, biostimulants, and bioelicitors (19, 33), probably explaining the increase in lettuce biomass. On the other hand, Biog process is the only one not trying to increase plant nutrition and beneficial microorganisms, but producing energetic compounds. However, several studies have reported this product´s biostimulant quality (3, 15). Accordingly, our results displayed an increase in plant biomass, showing no toxic effects at dilutions below 25%.

Inhibition of plant growth in early stages has been reported by the application of digestates, suggesting dilutions below 5% in these periods (Medina *et al.*, 2015), probably explaining the differences in plant biomass as regards Fert treatments. Consequently, the use of these products as stimulants in the initial stages of plant growth and development may be counterproductive, thus higher dilutions are needed. Another important factor to be considered is the substrate used. Previous studies demonstrated that compost combined with digestate is the best treatment for plant growth, even at a similar level or above commercial substrate and chemical fertilizers (14). All this suggests complex interactions among biostimulants, substrates, and plants, being important for phenologic stage, concentration, and frequency of product application.

Chlorophyll content is highly correlated to N content in leaves and may be used as a nutritional indicator (23). All digestates significantly increased this parameter, suggesting the nutritional beneficial effect, mainly N intake in lettuce and tomato plants. Although bioproduct composition presents macro and micronutrients, they also contribute with microorganisms that promote plant growth (18) by mechanisms like hormone production, nutrient solubilization, and N_2 fixation. This may explain the increase in plant biomass, despite the lower nutrient content with respect to inorganic fertilizer, indicating that in a bioproduct, microbial content may be more important than nutrient concentration.

Stress indicators Fv/Fm and PIabs, suggested no bioslurries negative effect on plant growth. In lettuce, those plants treated with inorganic fertilizer presented the lowest values, suggesting higher photosystem II damage with respect to digestates and water control.

According to the manufacturer (Hansatech Instruments Ltd.), Fv/Fm near 0.85 indicates healthy tissues. Therefore, tomato showed greater damage than lettuce since the values were lower, being control the most affected and suggesting nutritional limitations. PIabs represents plant capacity to respond to stress, being more sensitive than Fv/Fm for stress determinations (2). Despite the high variability between lettuce and tomato, PIabs was significantly increased by Fert, possibly given to higher nutrient intake. Despite the lack of significance, all bioslurries increased these parameters, suggesting an improvement in plant coping ability under biotic and abiotic stress.

Our results demonstrated that digestates can be used as biostimulants for plant growth, with different properties depending on the source and brewing method. Dilutions are needed, due to the toxicity of the pure product, especially for seedling production, which may require even lower concentrations. Further studies are needed to determine dilutions, appropriate moment and frequency of application, and the possibility of combining the different digestates for optimal plant growth, allowing for reduced synthetic products, with lower negative impacts and safer production strategies.

Conclusion

Digestates are valuable by-products, rich in nutrients and microorganisms for high-quality plant production. Each product presented different characteristics and effects on plant biomass, suggesting complex interactions, thus consequent possible complementation in their use. All digestates stimulated plant growth. Bfao showed the highest effect on tomatoes and lettuce biomass followed by Blac and Biog, constituting an adequate alternative for a cheaper, safer and low-impact strategy for crop growth. The biostimulants presented high nutrient content and no phytotoxic effects at concentrations of 10%, being an excellent strategy to treat organic residues while high-quality by-products are obtained. Further studies are needed to determine optimal brewing conditions, dilutions, raw materials and application techniques for producers. Moreover, Liquid biofertilizers should be used and evaluated, not only as an isolated practice but also within a set of sustainable crop management strategies.

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Metallic elements in foliar material and fruits of three tree species as bioindicators

Elementos metálicos en material foliar y frutos de tres especies arbóreas como bioindicadores

Jorge Alonso Alcalá Jáuregui [1], Juan C. Rodríguez Ortiz [1], Maria Flavia Filippini [2], Eduardo Martínez Carretero [3], Alejandra Hernández Montoya [1], Ángel Natanael Rojas Velázquez [1], Heriberto Méndez Cortés [1], Felix Alfredo Beltrán Morales [4]

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Abstract

This study aimed to determine the presence of metal elements in fruits and leaves of three tree species as bioindicators in San Luis Potosi, Mexico. Ninety fruit and 90 leaf samples (30 per tree species) were collected at five sites (agricultural, suburban, commercial and services, urban, and mining), using the site and the presence of *P. laevigata*, *S. molle*, and A. *farnesiana* as references. Total concentrations of 13 metal elements were determined using an inductively coupled plasma mass spectrometer (ICP-MS). The species were significantly affected by Co, As, and Pb (ANOVA, $p \leq 0.05$). Considering the land use and the species, the contents determined in the leaves and fruits of the three species had sufficient and phytotoxic contents of Al, Cd, Co, Ni, Mn, Ti, and Cr, and excessive ranges with the possible phytotoxic effect of As, Cu, Pb, Zn, V, and Fe. The *A. farnesiana* had the highest concentrations of 12 heavy metals analyzed (HM). The variation in total metal concentrations between leaves and fruits ranged from 84.70 to 99.06%, with V, As, and Cr being prominent. The functionality of these tree species as phytoremediators and bioindicators is reviewed to evaluate environmental impacts on land use.

Keywords
P. laevigata • *S. molle* • *A. farnesiana* • land uses • bioindicator

1 Universidad Autónoma de San Luis Potosí. Facultad de Agronomía y Veterinaria. Carretera San Luis Potosí - Matehuala Km. 14.5 Ejido Palma de la Cruz CP. 78321 Soledad de Graciano Sánchez. San Luis Potosí. México. Autor de correspondencia: jorge.alcala@uaslp.mx.
2 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Cátedra de Química Agrícola. Almirante Brown 500. M5528AHB. Chacras de Coria. Mendoza. Argentina.
3 IADIZA-CONICET, Mendoza. Geobotánica y Fitogeografía. Universidad Nacional de San Juan. Facultad de Ciencias. Exactas, Físicas y Naturales. Argentina.
4 Centro de Investigaciones Biológicas del Noroeste, Carretera a San Juan de La Costa El Comitán Km. 1, Playa Palo de Santa Rita Sur; La Paz, B.C.S. México. 23096.

Resumen

El objetivo del estudio fue determinar la presencia de elementos metálicos en frutos y hojas de tres especies arbóreas como bioindicadoras en San Luis Potosí, México. En cinco usos de suelo (agrícola, suburbano, comercio y servicios, urbano y minero) y tomando como referencia la ubicación y presencia de 30 árboles de *P. laevigata*, *S. molle* y *A. farnesiana.* Fueron colectadas en total 90 muestras de frutos y 90 de hojas (30 por especie). Las concentraciones totales de 13 elementos metálicos se determinaron utilizando un equipo de espectrometría de masas de plasma acoplado inductivamente (ICP-MS). La especie tuvo efecto significativo en Co, As y Pb (ANOVA, $p \leq 0,05$). Considerando los usos de suelo y la especie, los niveles determinados en hoja y fruto en las tres especies presentaron niveles suficientes y fitotóxicas en Al, Cd, Co, Ni, Mn, Ti y Cr y excesivos con posible efecto fitotóxico de As, Cu, Pb, Zn, V y Fe. *A. farnesiana* obtuvo las concentraciones más altas de 12 metales pesados (MP). La variación en las concentraciones totales de metales entre hoja a fruto osciló de 84.70 a 99.06%, destacando V, As y Cr. Se comprueba la funcionalidad de estas especies arbóreas como fitorremediadoras y bioindicadoras para evaluar el impacto ambiental en usos de suelo.

Palabras clave

P. laevigata • *S. molle* • *A. farnesiana* • usos de suelo • bioindicador

Introduction

Tree species represent a potential bioindicator for studying environmental pollutants due to their ability to accumulate heavy metals. Among the ecological services provided by vegetation, microenvironmental enhancement, runoff reduction, carbon sequestration and accumulation, noise reduction, air purification, and pollutant sequestration are essential (19). Heavy metals (HM) are of interest in environmental studies, and tree species serve as bioindicators (42). Plants have evolved mechanisms to take up and tolerate these potentially toxic elements (36). They accumulate readily and are persistent because they are not biodegradable or thermally degradable and can be toxic at high concentrations (21, 32). Some oligo-elements, such as Fe, Mn, Zn, Cu, Mg, Mo, and Ni, have biological significance, while others, such as Cd, Cr, Pb, Co, Ag, Se, and Hg, are phytotoxic (18, 28). Human activities such as car exhaust, industrial factories, urban waste, intensive use of fertilizers, and others (28, 29) are the primary sources of emissions. Vegetation can absorb HM through roots, stems, and leaves; however, accumulation depends on the specific metal element, plant species, and environmental conditions (15, 19). In addition, HM exhibits different behavior and mobility patterns in the plant, with some tending to immobilize and accumulate in the roots, while others migrate to the aboveground organs (15). In different regions of the world, interest in the study of HM is based on biological and phytogeographical characteristics of urban trees (19, 30).

In Mexico, anthropogenic activity, especially mining, is thought to have increased, contaminating with HM such as Hg, As, Pb, and Cr (17). San Luis Potosi (Mx) has sites contaminated with As and Pb (21). In the urban and suburban areas of San Luis Potosi and Graciano Sanchez, atmospheric particles contaminated with Fe, Pb, Cr, Mn, Ni, Cu, Cd, As, and Ti, among others, were found in soils, river sediments, and leaves and bark of trees (5, 6, 7, 8, 9, 12, 35). These data demonstrate the environmental impact of habitat fragmentation due to soil diversification and urban-industrial activities. The study is focused mainly on determining the concentrations of HM in plants, and in some cases, their hyperaccumulation potential was highlighted. However, there is a need to investigate the effects of HM on plant populations and the associated impacts on ecosystems and genetic and biological diversity (25). This work aimed to examine metallic elements in fruits and leaves of three common tree species in the urban area of San Luis Potosi (Mx).

Material and methods

The study area is located in the state of San Luis Potosi, in the urban-suburban area of the municipalities of San Luis Potosi (22°09’05” North, 100°58’37 West, 18640 m a.s.l.) and

Soledad de Graciano Sanchez (22°11’16” North, 100° 56’ 14” West, 1850 m a.s.l.) (23). The climate is semiarid (BS1k), very arid (BWk), and very dry semiarid (BWh), with an annual rainfall of 400-500 mm. The predominant soils are Vertisols, Durisol, and Leptosol (23). According to the urban and suburban dynamics between the cities of San Luis Potosí and Soledad de Graciano Sanchez, a road route was established and defined by the influence of five land uses, agriculture, urban development, commerce, services, and mining. For each land use, six points were determined considering the presence of the three tree species: *Prosopis laevigata* L. Willd. (Mesquite), *Acacia farnesiana* L. Willd. (Huizache) and *Schinus molle* L. (Pirul). Trees aligned along the road, considering a maximum distance of 10 m between individuals of each species. A total of 90 leaves and 90 fruit samples (30 for each material and tree species), with an average weight of about 50 g, were randomly selected on the left and right sides of branches with a height of 1.60 to 1.80 m. The samples were collected in July and August 2018 (figure 1). The collected material was washed with distilled water and dried in an oven at 60°C. From each collected material, 1 g was calcined to triplicate at 450° C, then the niter extract was prepared (nitric acid 1%) and filtered. The following elements were analyzed: Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb using the ICP-MS (inductively coupled plasma mass spectrometry) (5, 6, 7, 8, 9, 40). All the analyses were performed in the laboratories of Environmental Engineering and the Institute of Geology (UASLP, Mx). The results were expressed in mg kg^{-1} dry weight. In addition, the concentrations of HM in the leaves and fruits were used to calculate the difference between materials (mg kg^{-1} in dry weight) and the percentage variation in concentration between species. Data were statistically analyzed using ANOVA and Pearson correlation coefficient ($\alpha \leq 0.05$), including interactions among factors of soil use, species, and biological material (leaves and fruits). In addition, principal component analysis was performed considering 13 HM. All analyses were performed using MINITAB 16® statistical software.

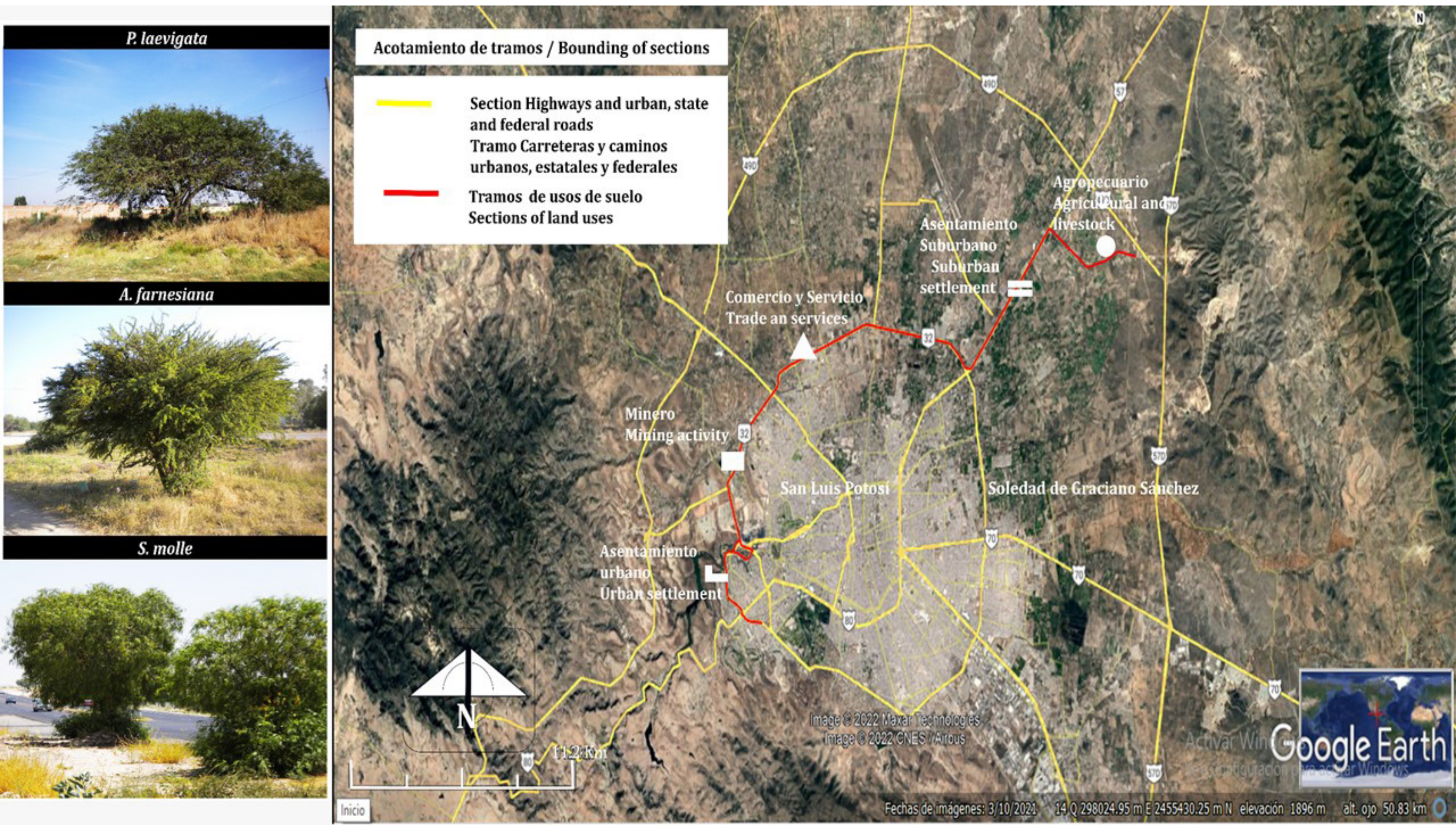

Figure 1. Location of the study area.

Figura 1. Localización del área de estudio.

Results and discussion

13 HM, evaluated in 180 samples, showed an average concentration ratio in leaves and fruits of the three tree species (mg kg^{-1} dry weight) oscillated at the ratio of Al > Fe > Zn > Mn > Pb > Cu > Ti > As > V > Ni > Cd > Cr > As. Correlation analysis revealed that 78 interactions were positively significant ($p \leq 0.05$, table 1). Of these, the highest values were for Co-Fe (r=0.979), Co-Mn (r=0.976), and Fe-Mn (r=0.973).

Table 1. Significant relations of the Pearson correlation coefficient in metallic elements assessed in leaves and fruits material of trees of the three species (p≤0.05, n=180).

Tabla 1. Relaciones significativas del coeficiente de correlación de Pearson en elementos metálicos evaluados en hojas y frutos de árboles de las tres especies (p≤0,05, n=180).

Elements	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd
Ti	0.433											
V	0.254	0.617										
Cr	0.242	0.947	0.636									
Mn	0.506	0.930	0.594	0.911								
Fe	0.459	0.966	0.645	0.963	0.973							
Co	0.376	0.930	0.638	0.954	0.976	0.979						
Ni	0.628	0.915	0.592	0.848	0.952	0.929	0.904					
Cu	0.423	0.853	0.518	0.783	0.724	0.785	0.731	0.803				
Zn	0.280	0.836	0.613	0.860	0.865	0.861	0.915	0.815	0.743			
As	0.618	0.793	0.511	0.702	0.896	0.823	0.839	0.891	0.569	0.754		
Cd	0.330	0.693	0.385	0.647	0.616	0.641	0.659	0.629	0.771	0.819	0.511	
Pb	0.819	0.561	0.375	0.448	0.687	0.613	0.601	0.771	0.583	0.585	0.764	0.531

The analysis of ANOVA showed no significant effect on the concentrations of HM in the leaves and fruits of the trees when the impact of land use was taken into account. However, the influence of tree species significantly affected only three HM of the 13 HM identified. In both cases, the non-significant and significant results indicate concentrations considered adequate or regular, toxic or excessively toxic according to various technical sources (table 2 and table 3, page 65). Regarding the non-significant concentrations of HM in the leaves and fruits of the samples from the different land use types, it is noticeable that the mean concentrations of As, Cu, Pb, Zn, V, and Fe are above the values considered sufficient or regular, toxic or excessively toxic (table 2, page 65). Likewise, it is noted that the mean concentrations of Co, Ni, Ti, Cr, Mn, and Fe in leaf material and fruits were higher in samples with urban use; and V, Cu, Zn, and Cd in samples with mining-industrial land use, in cases with phytotoxic levels (14, 15, 16, 24). Regarding the presence of Al and Pb, concentrations were higher in suburban land use, and As was more elevated in leaf and fruit samples from trees in agricultural land use. Similar studies using leaf material of the same species found that the concentrations of As, Co, and Pb were higher in trees located on mining land (6). Comparing the concentrations of the 13 elements in leaf and fruit samples of the three tree species studied, the effect was not-significant for Al, Ti, V, Zn, Cr, Mn, Fe, Ni, Cu, and Cd (table 3, page 65). For Co, As, and Pb, the significant concentrations depended on the tree species (table 4, page 66). First, it is noticeable that the ratio of the average concentrations of the metallic elements in the three tree species in leaves in the order Al > Fe > Zn > Mn > Pb > Cu > Ti > As > V > Cd > Ni > Cr > Co and in fruits Al > Fe > Zn > Mn > Ti > Cu > Pb > Ni > As > V > Cd > Cr > Co.

For the ten HM, where the concentrations presented in table 3 (page 65) were not-significant, values considered excessively phytotoxic were obtained for As, Cu, Pb, Zn, V, and Fe, with reference, values greater than 5-20 mg kg^{-1}, 20-100 mg kg^{-1}, 30-300 mg kg^{-1}, 100-400 mg kg^{-1}, and 5-10 mg kg^{-1} and > 500 mg kg^{-1}, respectively (14, 16, 24). These data indicate the influence of humans on metal elements. It has been shown that the spatial distribution of plant species, the location of point sources of pollution, and urban climatic factors (especially the wind factor) can be crucial in studying Cd, As, Pb, Cr, Cu, and Mn (39).

Table 2. Ratio of average concentrations of metal elements in fruits and leaves of the three tree species as a function of land use and reference values for normal and toxic levels (n=180).

Tabla 2. Relación de concentraciones medias de elementos metálicos en frutos-hojas de las tres especies arbóreas según los usos de suelo y referencia de niveles normales y tóxicos (n=180).

Land uses/ elements	Agricultural and livestock	Suburban settlement	Commercial and services	urban settlement	mining industry	Sufficient or Normal level	Toxic or Excessive toxic levels range
	Mean (mg kg^{-1})					mg kg^{-1}	
Al	986.48	1218.37	943.93	823.77	658.69	10-1000 (+)	NA
Ti	13.43	15.36	9.81	23.48	11.22	0.1-4.6 (+)	NA
V	4.81	4.43	3.36	13.9	16.66	0.27-4.2 (+)	0.5 (+)
Cr	100	1.31	0.70	2.79	1.09	0.1-0.5 (*) 0.02-0.2 (+)	*5-30 (*) >10 (+)
Mn	93.16	94.6	58.6	137.02	54.31	20-300 (+)	300-500 (+)
Fe	430.21	574.02	418.3	982.75	374.46	20-100 (+)	
Co	0.38	0.41	0.31	0.90	0.42	0.02-1 (*) 0.03-0.27 (+)	15-50 (*) 6-143 (+)
Ni	3.19	3.27	1.61	3.74	2.60	0.3-3.5 (+)	NA
Cu	19.70	26.94	16.19	30.02	32.94	*5-30 (*) 1-10 (+)	30-100 (*) 20-30 (+)
Zn	180.89	184.76	76.05	439.76	539.64	27-10 (*) 27-100 (+)	100-400 (*) 100-400 (+)
As	13.29	6.81	3.63	12.37	8.18	<0.5 (*) 0.009-1.7(+)	5-20 (*) > 2 (+)
Cd	1.26	3.38	0.51	3.06	5.83	0.05-0.02 (*) 0.1-1.0 (+)	*5-30 (*) >3 (+)
Pb	34.43	38.42	15.52	26.73	32.17	0.5-3 (*) 1.0 (+)	30-300 (*) >1(+)

(*) The Site and Soil Characterization Guide for Hazardous Waste Sites considers excessive or toxic areas in various plants (14). (+) The Trace Element Handbook (24) considers reference concentrations in multiple plants. NA. Data not available

(*) Guía de Descripción de Sitios y Suelos para la Caracterización de Sitios de Residuos Peligrosos considera rangos excesivos o tóxicos en varias plantas (14). (+) El manual de Oligoelementos (24) considera concentraciones de referencia en varias plantas. NA. Dato no disponible.

Table 3. Ratio of concentrations of non-significant metallic elements in leaves and fruits according to tree species and reference of average and phytotoxic values (n=180).

Tabla 3. Relación de concentraciones no significativas de elementos metálicos en hojas y frutos según especie arbórea y referencia de niveles normales y fitotóxicos (n=180).

Element/ Species	*S. molle*	*A. farnesiana*	*P. laevigata*		Sufficient or normal level	Toxic or Excessive toxic levels range
	Mean (mg kg^{-1})			SE Mean	(mg kg^{-1})	
Al	691,24	1094,75	992,75	210,074	10-1000 (+)	NA
Ti	15,43	11,53	17,02	4,617	0.1-4.6 (+)	NA
V	1,78	13,14	10,98	5,401	0.27- 4.2 (+)	0.5 (+)
Cr	1,04	1,16	1,93	0,659	0.1-0.5 (*) 0.02-0.2 (+)	*5-30 (*) >10 (+)
Mn	49,77	76,32	136,53	29,347	20-300 (+)	300-500 (+)
Fe	373,27	534,25	760,32	207,833	20-100 (+)	NA
Ni	2,24	2,86	3,6	0,707	0.3-3.5 (+)	NA
Cu	30,78	21,97	22,73	5,621	5-30 (*) 1-10 (+)	30-100 (*) 20-30 (+)
Zn	184,14	193,39	475,13	121,491	27-10 (*) 27-100 (+)	100–400 (*) 100-400 (+)
Cd	2,66	1,47	4,3	1,308	0.05-0.02 (*) 0.1-1.0 (+)	5-30 (*) >3 (+)

(*) The Site and Soil Characterization Guide for Hazardous Waste Sites considers excessive or toxic areas in various plants (14). (+) The Trace Element Handbook (24) considers reference concentrations in multiple plants. NA. Data not available

(*) La Guía de Descripción de Sitios y Suelos para la Caracterización de Sitios de Residuos Peligrosos considera rangos excesivos o tóxicos en varias plantas (14). (+) El manual de Oligoelementos (24) considera concentraciones de referencia en varias plantas. ND. Dato no disponible. NA. Dato no disponible.

(*) The Site and Soil Characterization Guide for Hazardous Waste Sites considers excessive or toxic areas in various plants (14). (+) The Trace Element Handbook (24) considers reference concentrations in multiple plants.

(*) La Guía de Descripción de Sitios y Suelos para la Caracterización de Sitios de Residuos Peligrosos considera rangos excesivos o tóxicos en varias plantas (14). (+) El manual de Oligoelementos (24) considera concentraciones de referencia en varias plantas.

Table 4. Ratio of significant Co, As, and Pb concentrations in three studied trees ($p \leq 0.05$).

Tabla 4. Relación de concentraciones significativas de Co, As y Pb en las tres especies de árboles ($p \leq 0{,}05$).

Elements	**Co**		**As**		**Pb**	
Species	Mean (mg kg^{-1})	SE Mean	Mean (mg kg^{-1})	SE Mean	Mean (mg kg^{-1})	SE Mean
Pirul tree	0,19	0,195	2.02 (+)	4,139	14.7(+)	5,429
Mesquite tree	0.42 (+)	0,195	7.33 (*+)	4,139	31.97(*+)	5,429
Huizache tree	0.84 (+)	0,195	17.22 (*+)	4,139	41.7(+)	5,429

Table 4 shows the relationship of the significant concentrations to the results of the substantial effect of Co (p=0.063), As (p=0.033), and Pb (p=0.002) and their toxicity reference. Co is naturally associated with rocks, soil, water, plants, and animals (2). Other sources include steel and metal industries, carbon combustion, and components for colored glass, ceramics, paints, and glazes (2, 19). On the other hand, Co is associated with delaying leaf senescence, increasing the drought resistance of seeds, and inhibiting ethylene biosynthesis, among others (33). As occurs naturally in soil and in the form of emissions from the steel and metal industries and coal and oil combustion (3, 19). As for Pb, plants can be contaminated by automobile exhaust, dust, and gasses from the steel and metal industry, oil, and coal combustion (4, 19, 37, 42). Higher significant concentrations of Co and As were found in leaves and fruits of huizache, with mean values of 0.84 mg kg^{-1} and 17.22 mg kg^{-1}, respectively (table 4); consequently, Co reached the toxic level and As reached excessive levels and phytotoxic (14, 24). Huizache was found to have a higher capacity to accumulate As, Pb, and Co in leaf material when used in mining soils (6). *Prosopis laevigata* could be a hyper-accumulating Pb species that responds in contaminated soils as a phytoremediator in arid and semiarid climates (13). In this study, Pb concentrations in huizache were higher at 41.7 mg kg^{-1}, which was within a toxic range (25, 27).

Intrinsic comparison of total HM in leaves and fruits revealed significant differences in the 13 elements evaluated. It is reported that Al, V, Cr, Fe, Co, Ni, Cu, Zn, As, Cd, and Pb had concentrations higher than those designated as regular, including excessive phytotoxic levels (table 5, page 67). In addition, concentration differences among materials (mg kg^{-1}) ranged from 84.70 to 99.06%. The data indicate a decrease in the concentrations of 10 elements: V 99.06%, As 98.33%, and Cr 98.15%. Usually, the concentration of HM is higher in leaves than in fruits of tree species (22). In the species studied, another factor is the timing of leaf and fruit maturity, as well as the possible differences between individual mesquite, huizache, and pirul species in terms of their biocapacity to concentrate and accumulate HM, as well as their availability to interfere with physiological processes in minimal amounts in some cases. Other studies indicate that the environmental impact of HM may occur in the biological cycle of plant leaves and fruits, as well as in other processes such as seed germination, growth and development, the decline in biomass production, the decline in flowering and fruiting, and yield reduction. This is because HM disrupts photosynthetic activity, interrupts nitrogen cycling and fixation, decreases chlorophyll content, causes deterioration of enzyme systems and intracellular mechanisms, and in some cases, impairs the uptake of other beneficial elements for plants (10, 20).

When analyzing the effects of the dual interaction between species and biological material (leaf-fruit) factors, a significant statistical relationship was found only for the concentrations of As and Pb (figure 2, page 68). The highest As value was found in samples of huizache, with 33.79 mg kg^{-1} in leaves and 0.65 mg kg^{-1} in fruits. Huizache showed the highest Pb concentration in leaves with 80.92 mg.kg-1 and in mesquite fruits with 3.57 mg kg^{-1}. *Acacia farnesiana* shows potential for phytostabilization and high Pb accumulation in roots, limiting translocation to new shoots (27); this follows the relationship stem >leaf > root (25), which is related to nitrogen in the soil and favors translocation to aboveground parts (25). These species show a higher accumulation capacity of As in roots, stems, and leaves (37). Consequently, huizache and mesquite are important bioindicator plants for contamination by HM (30). In many regions of San Luis Potosi, soil disturbance has resulted in high As and Pb concentrations (27).

Table 5. Ratio of significant concentrations of metal elements in leaves and fruits of the three studied species ($p \leq 0.05$, n=180).

Tabla 5. Relación de concentraciones significativas de elementos metálicos en hojas-frutos de las tres especies estudiadas ($p \leq 0,05$, n=180).

(*) The Site and Soil Characterization Guide for Hazardous Waste Sites considers excessive or toxic areas in various plants (14). (+) The Trace Element Handbook (1997) considers reference concentrations in multiple plants.

(*) La Guía de Descripción de Sitios y Suelos para la Caracterización de Sitios de Residuos Peligrosos considera rangos excesivos o tóxicos en varias plantas (14). (+) El manual de Oligoelementos (1997) considera concentraciones de referencia en varias plantas.

Elements		Material			
		Leaves	Fruits	Difference between materials (mg kg^{-1})	% concentration variation
		mg kg^{-1}	mg kg^{-1}		
Al	Mean	1815.48 (+)	37.01	1778.47	97.96
	SE Mean	171.25	171.25		
	Valor de P	0.000	0.000		
Ti	Mean	25.43 (+)	3.89	21.54	84.70
	SE Mean	3.77	3.77		
	Valor de P	0.000	0.000		
V	Mean	17.11 (+)	0.16	16.95	99.06
	SE Mean	4.10	4.10		
	Valor de P	0.007	0.007		
Cr	Mean	2.71 (+)	0.05	2.66	98.15
	SE Mean	0.53	0.53		
	Valor de P	0.001	0.001		
Mn	Mean	164.29	10.79	153.5	93.43
	SE Mean	23.962	23.962		
	Valor de P	0.000	0.000		
Fe	Mean	1085.17 (+)	26.72	1058.45	97.53
	SE Mean	179.695	179.695		
	Valor de P	0.000	0.000		
Co	Mean	0.930 (+)	0.040	0.89	89.93
	SE Mean	0.159	0.159		
	Valor de P	0.000	0.000		
Ni	Mean	5.21 (+)	0.590	4.62	88.67
	SE Mean	0.577	0.577		
	Valor de P	0.000	0.000		
Cu	Mean	46.98 (*+)	3.34	43.64	92.89
	SE Mean	4.590	4.590		
	Valor de P	0.000	0.000		
Zn	Mean	548.07 (*+)	20.37	527.7	96.28
	SE Mean	99.197	99.197		
	Valor de P	0.000	0.000		
As	Mean	17.42 (*+)	0.29	17.13	98.33
	SE Mean	3.379	3.379		
	Valor de P	0.000	0.000		
Cd	Mean	5.47 (*+)	0.15	5.32	97.25
	SE Mean	1.068	1.068		
	Valor de P	0.001	0.001		
Pb	Mean	56.63 (*+)	2.28 (+)	54.35	95.97
	SE Mean	4.433	4.433		
	Valor de P	0.000	0.000		

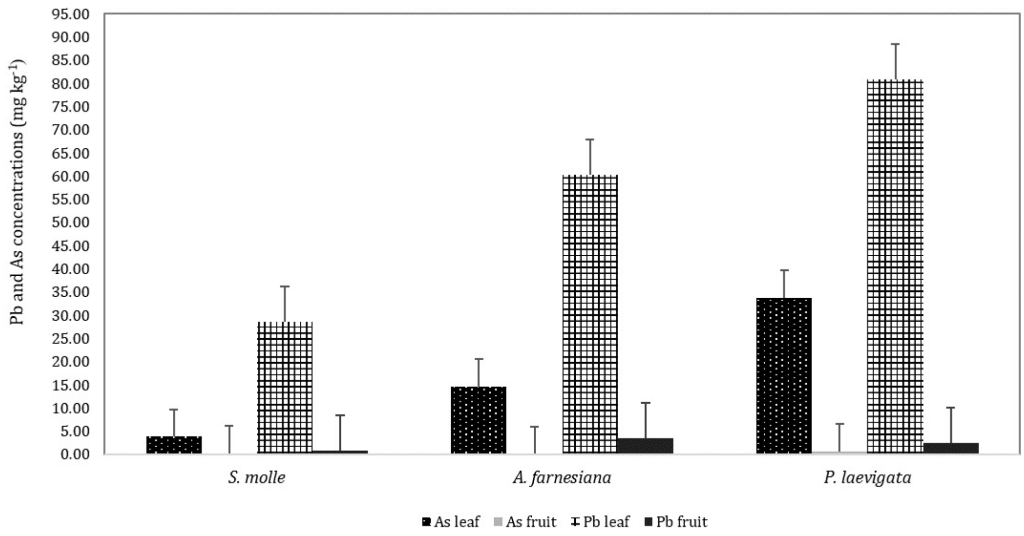

Figure 2. Comparative ratio of significant concentrations of As and Pb in the fruit and leaf of the three species studied.

Figura 2. Relación comparativa de concentraciones significativas de As y Pb en fruto y hoja de las tres especies estudiadas.

Principal component analysis shows that the first two components account for 84% of the total variance. In the PC1, the positive coefficient appears about Ti, Mn, Ni, and Co; in the PC2 of Al, Pb, and As and the negative in Cr, Zn, and V (figure 3, page 69). An isolated group refers to the leaves and fruits of huizache (figure 3BC, page 69).

Figure 3D (page 69) shows a group with samples of urban, mining, commercial and services land uses. Atmospheric particles are a source of HM, such as Fe, Mn, Cu, Zn, Pb, and Cd, and can be taken up by tree species (11). Differences in the content of heavy metals in the leaves of urban species have been found, including Pb, Zn, Cu, Cr, Ni, and Mn. It is noted that the elemental composition of plant leaves may differ in different systems of land-use-plant-atmosphere particles (26).

The similarity analysis revealed values ranging from 64.53% to 97.64% in the elements of leaves and fruits of three tree species (figure 4A, page 69). Chromium, Ni, Zn, As, Cu, and Cd have an 81-96% association. These data suggest an intrinsic species response to HM enrichment and serve as bioindicators (10, 19, 30, 31, 34, 36, 38). Vector analysis showed that the three determining factors explained 90% of the variance, with a significant relationship between metal elements in leaves and fruits of the three species according to land use (figure 4B, page 69).

Natural and anthropogenic factors influence the presence of HM in the environment. The latter include vehicle emissions, corrosion of metal parts, industrial activities, and construction materials. Factors such as climate, precipitation, wind direction and speed, and soil characteristics are involved in dispersion (1, 41).

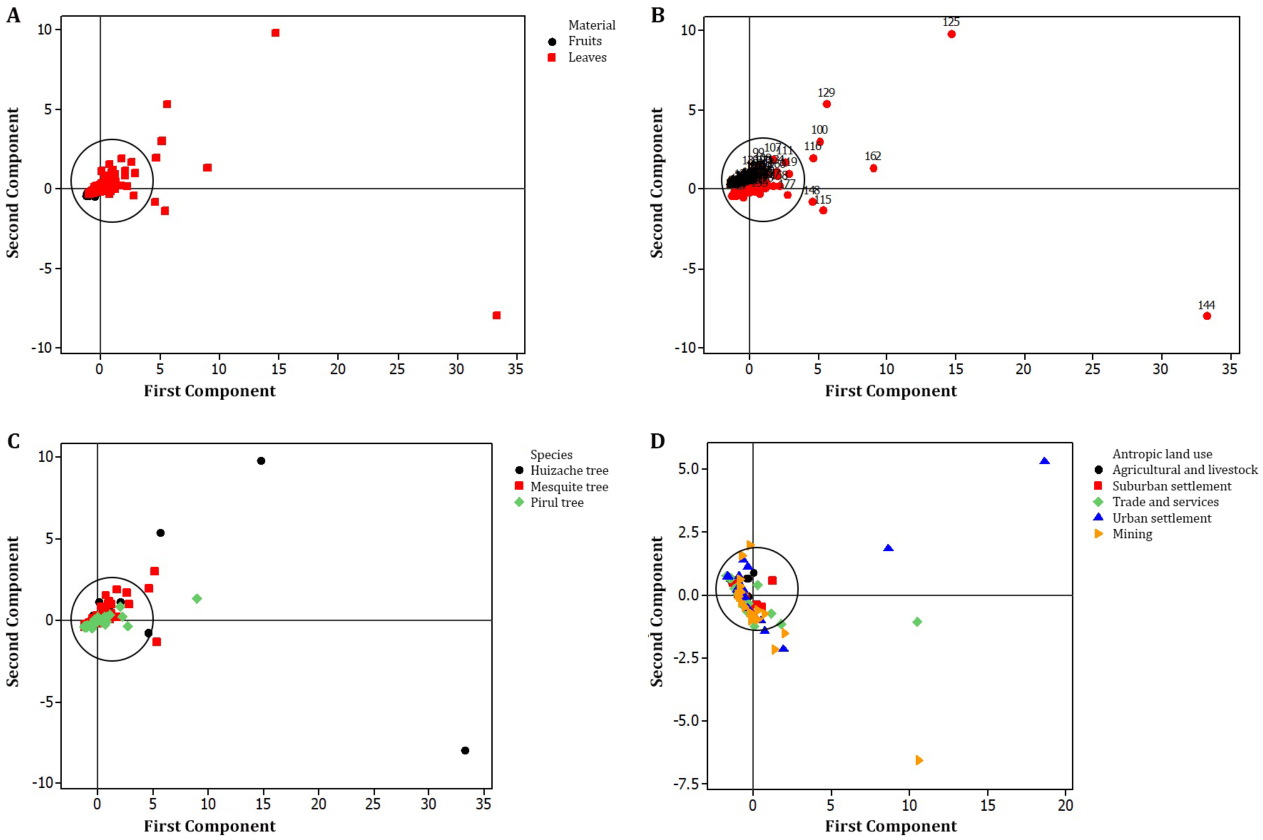

Figure 3. Eigenvalue distribution of metallic elements in (A) leaves and fruits, (B) number of samples, (C) species, and (D) land use resulting from principal components analysis.

Figura 3. Distribución eigenvalores de los elementos metálicos en A) hojas y frutos, B) número de muestras, C) especies y D) uso de suelo resultante del análisis de componentes principales.

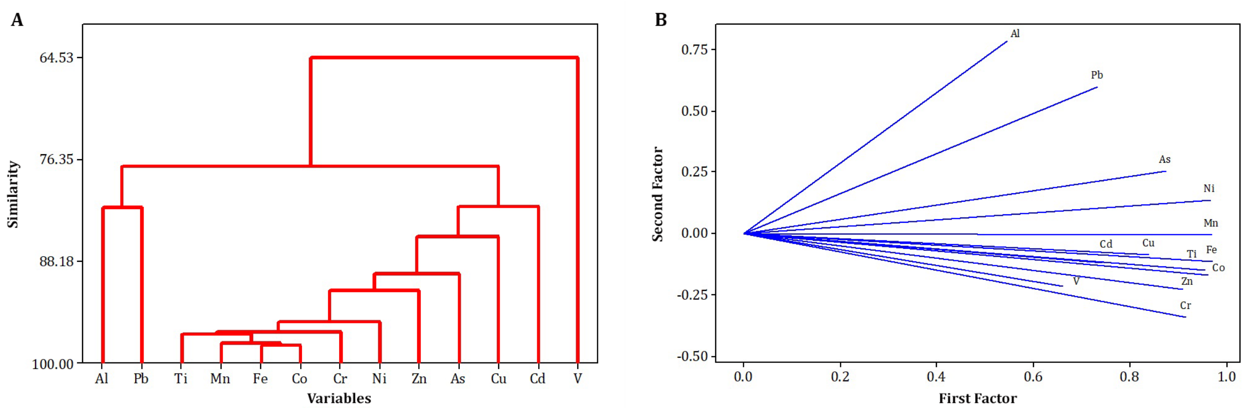

Figure 4. Dendrogram (A) and vectors (B) from component analysis of metallic elements in leaves and fruits of the three tree species.

Figura 4. Dendrograma (A) y vectores (B) derivados del análisis de componentes en elementos metálicos de hojas y frutos de los árboles de las tres especies.

Conclusion

Three tree species evaluated in the five land use factors showed bioindication potential through the concentration of HM in their leaves and fruits. Although the land use factor did not have a significant effect on the concentrations of HM, it can be said that the three species located in agricultural, livestock, suburban, commercial and services, urban, and mining lands had normal, toxic, and excessively phytotoxic levels in at least 12 elements. The factor species significantly influences Co, As, and Pb levels. The huizache, the species with the highest concentrations in leaves and fruits, and others in 9 elements that were not significant; however, the levels determined were normal, toxic, and excessively toxic. In the intrinsic relation of leaf and fruit, the concentrations of 10 elements were reduced by more than 90% from one material to another. Al, Cd, Co, V, Ni, Ti, Cr, As, Cu, Pb, Zn, V, and Fe reached toxic concentrations. These results demonstrate the value of plant species as bioindicators and their potential use in phytoremediation measures under different land use conditions in the studied region.

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Management improvement of the supply chain of perishable agricultural products by combining the Scor model and AHP methodology. The ecuadorian flower industry as a case study

Mejorar la gestión de la cadena de suministro de productos perecederos agrícolas combinando el modelo Scor y la metodología AHP. La industria floral ecuatoriana como caso de estudio

Luis Rodríguez-Mañay [1], Inmaculada Marques-Perez [2*], Inmaculada Guaita-Pradas [2]

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Abstract

This research aims to identify and propose an analysis and redesign methodology for Supply Chain (SC) processes, leading to better performance and financial results. Our study focuses on the Ecuadorian flower industry redesigning processes and allowing higher levels of competitiveness. The methodology here proposed combines the SCOR (Supply Chain Operation Reference) and a Multi-Criteria Evaluation methodology, the Analytic Hierarchy Process (AHP). The SCOR model allows mapping and describing the supply chain. By consulting with experts, the AHP helps examine and select decisive chain operational aspects for successful performance allowing redesign. According to the proposed methodology and expert consultation, those metrics, attributes, and processes with lower weight, should be improved. Although few research articles have applied the SCOR and AHP models to the agricultural sector, this study on the supply chain of the Ecuadorian floriculture sector leads us to conclude that model combination is a suitable methodology for supply chain analysis of any perishable product and, more specifically, the flower industry.

Keywords

AHP • SCOR • supply chain • agri-food management

1 Universidad Central del Ecuador. Facultad de Ciencias Administrativas. Quito 170129. Ecuador.

2 Universidad Politécnica de Valencia. Facultad de Administración y Dirección de Empresas. Departamento de Economía y Ciencias Sociales. 46022 Valencia. España.

[*] imarques@esp.upv.es

Resumen

Esta investigación tiene como objetivo identificar y proponer una metodología para analizar y rediseñar los procesos de la Cadena de Suministro (CS), lo que conduce a un mejor rendimiento y, por tanto, a mejores resultados financieros. Nuestro estudio se centra en la industria ecuatoriana de las flores para impulsar el rediseño de estos procesos que le permitan alcanzar mayores niveles de competitividad. La metodología aquí propuesta combina el SCOR (Supply Chain Operation Reference) y una metodología de Evaluación Multicriterio, Analytic Hierarchy Process (AHP). El modelo SCOR permite mapear y describir la cadena de suministro y, mediante la consulta a expertos, el AHP ayuda a examinar y seleccionar aquellos aspectos operativos de la cadena que son decisivos para su buen funcionamiento y que, por tanto, deben ser rediseñados. De acuerdo con la metodología propuesta y la consulta a los expertos, deben mejorarse aquellas métricas, atributos y procesos que obtuvieron una menor ponderación. Aunque son pocos los artículos de investigación que han aplicado los modelos SCOR y AHP al sector agrícola, este estudio sobre la cadena de suministro del sector florícola ecuatoriano nos lleva a concluir que la combinación de ambos es una metodología adecuada para analizar la cadena de suministro de cualquier sector de productos perecederos y más concretamente la cadena de suministro de flores.

Palabras clave

AHP • SCOR • cadena de suministro • gestión agroalimentaria

Introduction

Given that flowers are perishable and temperature-sensitive products, using a cold supply chain (SC) is imperative for avoiding financial losses (7, 19). In cut flower production, supply chain management is key for business design (19, 30). In this sense, optimization levels and SC best practices in the flower industry need to improve production efficiency and distribution.

The supply chain management (SCM) concept coordinates the different corporate partners, internal departments, processes, and customers along a supply chain (6, 31). Supply chain integration allows gaining competitive advantages through SCM, involving internal integration through effectively exchanging information with customers and suppliers. By achieving integration, the SC functions as one single unit directly driven by customer demand (12, 18, 26). In this sense, several models have been developed to measure SC performance, and the SCOR model stands as a powerful tool to evaluate SC's activities and performance, optimizing production, distribution, and sales processes (1, 17).

The SCOR model was developed by the Supply Chain Council (SCC) in 1996 with the intention of understanding, describing, and assessing supply chains. This model provided a general framework, as well as standard terminology, common metrics, and best practices (22, 34). The SCOR model follows a hierarchical structure with different levels in the supply chain and a basic structure comprising three levels. This model may help understand a particular supply chain by mapping it in terms of the business processes (22, 29, 34). After selecting the appropriate process type, the configuration that best fits the supply chain is finally chosen. Application complexity depends on the type of product, demand, data reliability, and geographical distribution of both customers and suppliers (9, 39).

The multicriteria methodology Analytic Hierarchy Process (AHP) structures complex decisions into hierarchies, translating goals into measurable criteria and sub-criteria, which, in turn, can lead to alternative decisions. It assigns priority to each hierarchy level. Alternative priorities are then compared with those of the criteria determining alternative final importance (4, 5, 33). Saaty and Vargas (2012) suggested that each group member makes individual pairwise comparisons and preference judgments about the alternatives, establishing group priorities. Thus, the individual preference geometric means allow calculating a preference matrix establishing group priorities (27). The combination of AHP (33) and the SCOR model, together with experts in the field, could accurately determine the most important processes of the SC given the specific product and company, establishing process efficiency.

Our study tests both SCOR and AHP models to analyze the SC of the flower industry, identifying those processes to be redesigned, achieving higher levels of competitiveness. Regarding the Ecuadorian flower industry and given the shortcomings in competitiveness and logistics performance, we decided to apply the study to the Ecuadorian flower sector, as a case study.

With an encompassing purpose of research at sector level, Ecuadorian companies and organizations related to flower production and market were considered. In this regard, various authors have already studied the floriculture sector SC. Villagrán *et al.* (2021) designed the structure of the supply chain management for the Colombian flower sector. Verdouw *et al.* (2013) explored the virtualization of the floriculture supply chain in the Netherlands and Janssen *et al.* (2016) focused on collaboration in the Dutch flower sector supply chain. Meanwhile, the African floriculture supply chain was examined by Button (2020), and recently, Karpun *et al.* (2020) developed a conceptual model for flower supply chain management.

Flowers are the fourth export product in Ecuador, after oil, bananas, and shrimp. From 2014 to 2018, exports of cut flowers to different destinations reached an average value of about USD 800 million. However, few studies approach supply chain analysis (Mendonza Lima *et al.*, 2021 and Tagarakis *et al.*, 2021). These authors suggested introducing a traceability system optimizing time, money, personnel, internal communication and, of course, guaranteeing flower quality. According to Herrera-Granda *et al.* (2020), implementing the SCOR model in the production process of flower companies would improve end-costumer services.

Our research intends to benefit different areas: Economically, this study will enable the human, material, and technological resources to be optimized and controlled. Concerning supply technology, our study may assist in the creation of a process/ performance monitoring application. Consequently, this knowledge will allow for a more efficient activity, leading to a more significant market share in the European Union, which is currently at 4%. In the case of Ecuador, the methodology proposed could also be applied to other major sectors of the Ecuadorian economy, such as the shrimp and banana sectors.

Materials and methods

Supply Chain Operations Reference (SCOR)

The SCOR model structure and the interrelationship among processes were confirmed by Zhou *et al*. (2011). The performance attributes serve to define generic supply chain characteristics and to describe the supply chain strategy. The SCOR model metrics are organized around the performance attributes and have different hierarchical levels, in the same way as SCOR processes (22, 34, 41).

Given that when establishing the relevant processes, only those belonging to levels 1 and 2 of the SCOR model are used, our analysis uses only level 1 performance attributes and metrics for selecting the target process (22, 34).

Analytic Hierarchy Process (AHP)

The pairwise comparisons are made per hierarchy levels, and each stakeholder must compare and decide which factor is more important for each level, according to the Saaty scale (33) From these comparisons, positive reciprocal matrices are obtained. A Saaty matrix (equation 1) is then created for each of the decision-makers, A_k, where a_{ij} is the result of the comparison between factor *i* and factor *j* of the hierarchy:

$$A_k = \begin{bmatrix} a_{11} & a_{12} \cdots & a_{1n} \\ a_{21} & a_{22} \cdots & a_{2n} \\ . & . & . \\ . & . & . \\ . & . & . \\ a_{n1} & a_{n2} \cdots & a_{nn} \end{bmatrix} \quad (1)$$

Using the matrices with the individual preferences, the priorities of each stakeholder are calculated (equation 2) according to the different levels and following the eigenvector method (EM): weights vector is the eigenvector corresponding to the maximum eigenvalue of matrix A.

$$A \times W = \lambda_{max} \times W \tag{2}$$

where
A = the preference matrix
W = the vector of priority or vector of weights
λ_{max} = the maximum eigenvalue of the matrix.

Prioritization results can be seen in the following equation:

$$W = (w_1, w_2, \dots, w_n)^k \tag{3}$$

However, not all the comparison matrices can be included in the results. Firstly, preference consistency expressed by each decision maker is verified to confirm valid individual opinions for group priorities. This consistency can be checked through a consistency analysis, calculating the Saaty consistency index (CI) for each preference matrix (equation 4).

$$CI = \frac{\lambda_{max} - n}{n - 1} \tag{4}$$

The consistency ratio (CR) is calculated from CI and is defined as the CI to RI ratio:

$$CR = \frac{CI}{RI} \tag{5}$$

where
RI = the mean *CI* value of the pairwise comparisons of randomly obtained matrices of the same order. For *CR* under 10% (0.1), the matrix is considered consistent.

Our analysis of the flower industry supply chain intends to identify the SC processes to be improved or redesigned according to performance attributes and the metrics used to measure each attribute. Figure 1 shows the level selection of the "to be redesigned processes". On the second level, performance attributes, followed by metrics for each attribute, and finally, on the lowest level, the model supply chain processes.

Source: Authors', based on a proposal by Palma-Mendoza (2014).
Fuente: Elaboración propia, basado en la propuesta de Palma-Mendoza (2014).

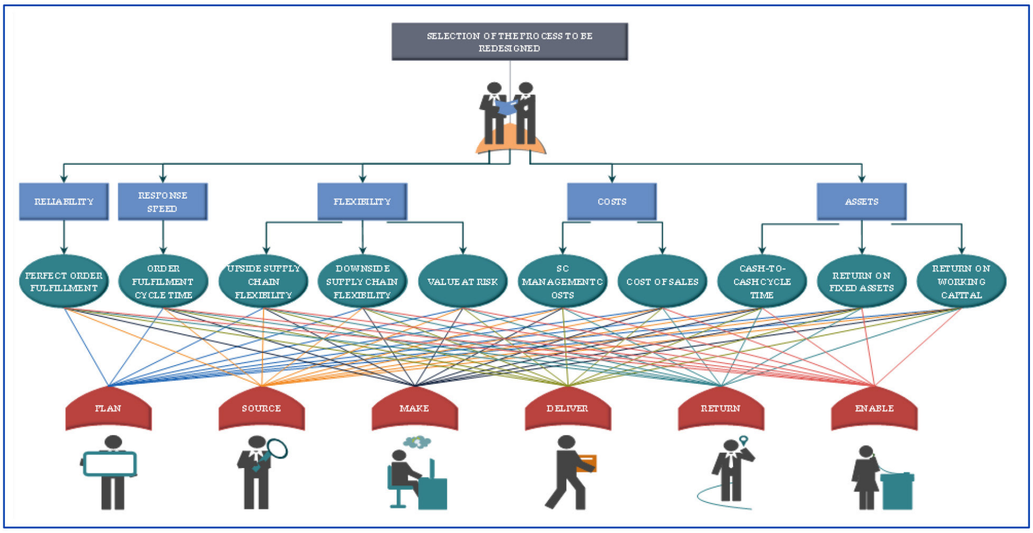

Figure 1. SCOR model mapping for redesigning processes.
Figura 1. Mapeo del modelo SCOR para el rediseño de los procesos.

Finally, through the AHP analysis, each factor in the hierarchy is weighed. and the order of importance already established by the experts are clearly visualized. This makes it possible to identify those attributes, metrics, or processes that may initially have been less considered but, according to expert opinion, should receive greater attention.

Data on stakeholder preferences can be gathered through an online survey, using a specific digital questionnaire designed for this purpose. The questionnaire should be user-friendly and facilitate reflection and decision-making. Content, structure, and design are essential components, and the respondent should be able to answer individually and share his or her personal experience. Besides the questions, it should also include descriptions of decision-making in the floriculture sector, the AHP hierarchy, the Saaty scale, and how to make pairwise comparisons. Stakeholders are invited to share their expert opinion through the online survey (23).

Case study

Flower production in the Ecuadorian Pichincha and Cotopaxi provinces account for 83% of the production, with the largest number of flower companies. This study gathered, a group of experts in the flower industry representing the largest 100 Ecuadorian flower companies (order established based on the income data published by the Superintendencia de Compañías del Ecuador), accounting for approximately 80% of the industry's turnover in 2019 (2, 8). The group was also integrated by academics from the Facultad de Administración de Empresas de la Universidad Central del Ecuador; government experts in floriculture from the municipality of Cayambe; and flower quality control specialists. All participants were given equal importance in the decision-making process (24).

A digital questionnaire gathered information on stakeholder preferences (https://docs.google.com/forms/d/1YzlailVXXF0xk4tURIM3v1GweL2oINS3RMh_WdG-F7Q/edit?usp=drive_web).

The questionnaire was divided into four sections:

- The first section described objectives and requested information on company or institution identity. It also included information on the Ecuadorian flower sector, the Ecuadorian supply chain and the AHP hierarchy with the objective of redesigning elements, metrics, and processes. Finally, it added an explanation of the Saaty scale for comparisons.
- The second section listed 10 questions related to pairwise comparisons of the supply chain processes for rank determination.
- The third section presented questions regarding metrics for each attribute (7 questions).
- Finally, the fourth section included 10 questions about performance metrics relevance.

Results and discussion

Several authors have applied this approach. Specifically, in their study into the Turkish clothing industry, Aydın *et al.* (2014) used the SCOR levels as follows: Level 1 described model scope and content, and in level 2, the company's supply chain was broken down into 26 process categories. The scope of the research focused on level 1 performance attributes and metrics. Meanwhile, Lhassan *et al.* (2018) considered level 1 as the strategic level at which the different supply chain processes and the role of the SC actors were defined. Among the actors considered were manufacturers, suppliers, wholesale distributors, and first and second-level customers. The distributing processes identified were plan, source, delivery, and return. At level 2, considered the tactical level, each level 1 process was broken down into two or more sub-processes. The questionnaire was sent to ninety-six companies, but only six answered with Hilsea Investments first in the ranking by income. In addition, answers were received from two academics, a local council official from Cayambe, and an expert in flower quality control for the Ecuadorian flower sector. A total of ten out of 100 submissions were answered.

The different stakeholder assessments showed coherence as well as acceptable consistency. Table 1 (page 78), presents level 1 weightings of the Ecuadorian flower supply chain.

Table 1. Level 1 metrics weights.
Tabla 1. Pesos para las métricas de nivel 1.

N°	Attribute Code	1st Level metrics	Code 1st level	Weight
1	RL	Perfect order fulfilment	RL11	0.4164
2	CO	Total supply chain management cost	CO11	0.1751
3	RS	Order fulfilment cycle time	RS11	0.1278
4	AG	Upside supply chain adaptability	AG11	0.0643
5	CO	Cost of goods sold	CO12	0.0556
6	AM	Cash-to-cash cycle time	AM11	0.0483
7	AG	Downside supply chain adaptability	AG12	0.037
8	AG	Value at risk	AG13	0.0369
9	AM	Return on supply chain fixed assets	AM12	0.0249
10	AM	Return on working capital	AM13	0.0137

Source: Author calculations.
*Attribute: Reliability (RL), Responsiveness (RS), Agility (AG), Cost (CO), Asset Management Efficiency (AM).
Fuente: Elaboración propia. *Atributos: Fiabilidad (F), Velocidad de respuesta (VR), Agilidad (AG), Coste (CO), Eficiencia en la gestión de activos (E).

Regarding the ten metrics proposed by the SCOR model, the results show that stakeholders considered the Perfect order fulfilment metric to be the most relevant, weighing 40%. Other significant metrics, albeit of less importance, are SC management cost, with 18%, and Order fulfilment cycle time, with almost 13%. The other seven metrics, only accounting for 29%, were considered unimportant by the experts. In decreasing order, these seven metrics are: Upside supply chain adaptability, Cost of goods sold, Cash-to-cash cycle time, Downside supply chain adaptability, Value at risk, Return on supply chain fixed assets, and Return on working capital.

Concerning the Upside supply chain adaptability metric, with 6% weight, the maximum period (for a company to adapt) suggested by the SCOR model, is 30 days. But in the case of the flower industry, this would be unfeasible, since depending on the species, it takes approximately twenty weeks for flowers to reach harvest time (11, 13, 22, 34) and, therefore, any adjustment would need more time. The fact that the flower industry has a limited capacity to quickly react to changes in demand may well be the reason for the low weight given to this metric. However, considering the outlook for the flower industry, it does seem that the Ecuadorian floriculture sector should pay greater attention to this SCOR supply chain metric. This is given the growing demand already observed during the first months of 2021 (5% increase as compared to the same period in 2020)(37), and also increasing exports predicted by the International Association of Horticultural Producers (AIHP). This organization estimates that flower demand in China will reach EUR 100 billion by 2030 (10).

In 2019 goods sold in the flower industry accounted for approximately 99% of the sales, one point higher than in previous years, whose average was 98% (2, 8). These percentages leave companies very little room for manoeuvre when it comes to establishing new markets or pricing strategies. This would explain the weight given to this metric (5.6%), which ranked fifth among the ten metrics studied. Despite the apparent need to optimize processes and production costs in the Ecuadorian flower sector, to our knowledge, there is no state-of-the-art research on this issue.

The experts participating in this study attached little importance to the Cash-to-cash cycle time metric, with a weight of less than 5%, ranking sixth. However, if this metric were better managed, flower companies could obtain annual surpluses, enabling them to invest further and improve their yield and production management. Based on the financial statements of some of the companies (2, 8), we estimated the cash-to-cash time cycle length at about 42 days. This is because the sum of the days of accounts receivable plus the days of inventory generates a relationship of 3 to 1 with the days of accounts payable. The average collection period was estimated at approximately 44 days. If this number of days were to be reduced to thirty, firms could then produce an annual surplus of up to USD 1,500,000 that could be invested in other asset types.

The SCOR model suggests 30 days for the *Downside supply chain adaptability* metric. As in the case of the *Upside supply chain adaptability* metric, the flower production system itself makes it challenging to meet these deadlines since the process cannot be suspended at short notice. Thus, the fact that this metric is difficult to manage and control may be the reason why it stands seventh with little attached importance.

The Value at-risk metric ranks eighth and is considered by floriculture organizations as part of the risk management function and not as a risk quantifying metric (32). Uncertainty is obviously inherent to the flower industry, making it extremely complicated for companies to forecast risks. From the flower companies' financial statements (published by the Superintendencia de Compañías del Ecuador), it was estimated that industry yield risk was about 44% in the 2015-2019 period (Expected return, $\bar{R}$; standard deviation, σ; coefficient of variation, CV; $CV = \sigma/\bar{R}$). This clearly indicates high risk, given that in the Ecuadorian floriculture sector, as previously mentioned, yield with respect to income is about 1%.

The Return on supply chain fixed assets metric (21) barely represents 2% of the total weight. This low percentage is due to the reduced margin of these flower companies, which in turn is related to the supply chain assets, and as previously stated, the high cost of sales leaves these firms little room for manoeuvre.

Finally, the Return on working capital metric (36) ranks last in level 1. The metric value calculated from the aforementioned financial statements data (Superintendencia de Compañías del Ecuador) was 10% (Return of working capital=(Revenue-Costs)/(Inventory+Accounts receivable-Accounts payable). This value is obtained after considering: (a) that the ratio between accounts receivable and inventory to the payable accounts is 3 to 1, and (b) given the reduced margin (1%) from the revenue minus total costs.

Table 2 offers both the calculated and the suggested performance weights of the Ecuadorian flower industry supply chain. Attributes with the largest gaps can be found in reliability, effective SC asset management, response speed, and flexibility.

Table 2. Calculated *vs.* suggested weights for the performance attributes.

Tabla 2. Pesos calculados *vs.* sugeridos para los atributos de desempeño.

Attributes	Calculated weights	Suggested weights	Difference	Percentage
Reliability	0.4164	0.2000	0.2164	108%
Costs	0.2307	0.2000	0.0307	15%
Agility	0.1382	0.2000	-0.0618	-31%
Responsiveness	0.1278	0.2000	-0.0722	-36%
Asset Management Efficiency	0.0869	0.2000	-0.1131	-57%

Source: Author calculations. * Calculated weight of the attributes - Suggested attribute weight = Difference = Gap.

Fuente: Cálculo de los autores * Peso calculado de los atributos - Peso sugerido de los atributos = Diferencia = Brecha.

Table 3 displays weights attributed to the six main supply chain processes (Plan, Source, Make, Deliver, Return and Enable).

Table 3. Process weight.

Tabla 3. Pesos de los procesos.

N°	Processes	Weight
1	Plan	0.3665
2	Source	0.1797
3	Make	0.1570
4	Deliver	0.1250
5	Return	0.0766
6	Enable	0.0952

Source: Author calculations.

Fuente: Elaboración propia.

These results show that the return and enable processes have the lowest scores, and should, in consequence, be more attentively observed. The return process is carried out by the flower companies themselves, but not following SCOR recommendations. In fact, the participating Ecuadorian flower companies did not specify protocols in relation to product reverse flow, nor did they indicate any aspects associated with return delivery scheduling, shipment and reception, all of which should be considered in the return process according to the SCOR model.

The enabling process showed the lowest weight, meaning no activity related to the SC management is carried out as recommended by SCOR. This implies no monitoring of trade rules, performance, data processing, resources, facilities, contracts, network supply chain, rule compliance, risks, or procurement.

Conclusions

We conclude that using both the SCOR model and AHP may not only constitute an appropriate methodology supply chain analysis of the floriculture sector but may also be applied to any other producing sector. To date, few research articles have combined the application of the SCOR and AHP methods to the agricultural sector.

The SCOR model permits to map and describe the supply chain, and along with experts and the AHP technique, identify and redesign those crucial chain aspects.

Performance attributes and metrics of the SCOR model cover all possible metric combinations measuring supply chain performance. Best practices recommended by SCOR apply to any supply chain structure.

In this study, level 1 metrics, SCOR attributes and all processes defined, were analyzed through surveys. Based on the pairwise comparisons in AHP, experts identified the most critical performance aspects needing to be redesigned.

According to the results obtained, improvements should focus on those lower-weighted aspects by applying the best SCOR practices. Concerning metrics, those to be improved are increasing and decreasing the supply chain adaptability, cost of sold goods, cash-to-cash cycle time, value at risk, return on supply chain fixed assets, and return on working capital. As for the attributes, the following need to be upgraded: reliability, supply chain asset management, responsiveness, and agility. Finally, regarding the processes, adjustments should focus on the return and enable (management) processes.

It is suggested that representatives of the Ecuadorian flower industry adopt the following measures: (a) continuous monitoring of demand behaviour, (b) reduction in the cost of sales share with respect to income, (c) reduction in number of days of receivable accounts and inventory, (d) risks monitoring risk management tools usage, (e) fixed assets usage optimization, and (f) reverse logistics application.

Several Ecuadorian flower companies took part in our study. But the participation of the flower export trade association as a whole, together with a larger number of Ecuadorian flower companies, could help obtain a more complete picture in future studies. Furthermore applying other approaches, such as business process reengineering (BPR) could help redesign processes.

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Sequential application of herbicide options for controlling *Conyza sumatrensis* in soybean pre-sowing

Aplicación secuencial de opciones de herbicidas para el control de *Conyza sumatrensis* en presiembra de soja

Leandro Paiola Albrecht [1], Alfredo Junior Paiola Albrecht [1], André Felipe Moreira Silva [2*], Romulo Augusto Ramos [3], Karine Yone Rodrigues da Costa [1], Gabriel Viana de Araújo [1], Tamara Thaís Mundt [1], Corina Colombari [1]

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Abstract

The aim of this study was to evaluate the efficacy of sequentially applied herbicides to control *Conyza sumatrensis*, one of the most widely distributed weeds worldwide, in soybean pre-sowing burndown. The study was conducted under field conditions in the state of Paraná, Brazil, at 2018-2019 growing season. The experiment consisted of a randomized block design with four replicates, with 12 treatments consisting of different herbicide mixtures applied before sowing. Control of *C. sumatrensis*, injury to soybean plants, and variables related to agronomic performance were evaluated. The control levels were high for all treatments, except for the one that was free of saflufenacil in either of the two applications. These results highlight the importance of saflufenacil in the control of *C. sumatrensis* and show promise for the use of saflufenacil/imazethapyr when considering the system and other weeds. All studied treatments were selective to soybean, which showed higher injury values in the presence of diclosulam; however, this did not compromise the agronomic performance of soybean.

Keywords

ALS inhibitors • *Glycine max* • PROTOX inhibitors • sumatran fleabane • weeds

1 Federal University of Paraná. R. Pioneiro. 2153. Jardim Dallas. 85950-000. Palotina. PR. Brazil.

2 Crop Science Ltda. Linha Bem-ti-vi. mailbox 01, 85955-000. Maripá. PR. Brazil.
* afmoreirasilva@hotmail.com

3 Basf S.A. Estrada Municipal Jose Lopes. 13833-612. Santo Antônio de Posse. SP. Brazil.

Resumen

El objetivo de este estudio fue evaluar la eficacia de los herbicidas aplicados secuencialmente para controlar *Conyza sumatrensis*, una de las malezas más ampliamente distribuidas en todo el mundo, en la pre siembra de soja. El estudio se realizó en condiciones de campo en el estado de Paraná, Brasil, en la campaña 2018-2019. El experimento consistió en un diseño de bloques al azar con cuatro repeticiones, con 12 tratamientos compuestos por diferentes mezclas de herbicidas aplicados antes de la siembra. Se evaluó el control de *C. sumatrensis*, daño a plantas de soya y variables relacionadas con el comportamiento agronómico. Los niveles de control fueron altos para todos los tratamientos, excepto para el que estaba libre de saflufenacil en cualquiera de las dos aplicaciones. Estos resultados resaltan la importancia de *saflufenacil* en el control de *C. sumatrensis* y son prometedores para el uso de *saflufenacil/imazethapyr* cuando se considera el sistema y otras malezas. Todos los tratamientos estudiados fueron selectivos a la soja, que mostró mayores valores de daño en presencia de *diclosulam*; sin embargo, esto no comprometió el comportamiento agronómico de la soja.

Palabras clave

inhibidores de la ALS • *Glycine max* • inhibidores de la PROTOX • rama negra • malezas

Introduction

The hairy fleabane (*Conyza bonariensis*), horseweed (*Conyza canadensis*), and the Sumatran fleabane (*Conyza sumatrensis*) are among the most dominant weeds found worldwide. *C. sumatrensis* is believed to have originated in subtropical South America and subsequently dispersed to Europe, America, and Asia (11). It is an herbaceous plant with an annual life cycle and high seed production (9, 14), and it is found in various agricultural environments, such as grain crop fields (18). *Conyza* spp. disperses exclusively through seeds present in the achene. The number of seeds produced by a single plant varies from 100,000 to 200,000 (9). For *C. sumatrensis*, this number can be as high as 350,000 seeds produced during the plant cycle These seeds are positively photoblastic, and thus germinate only in the presence of light, a feature favored by direct sowing in straw, at temperatures below 28-30°C. The seeds germinate mainly from fall to early spring (33) in the off-season, before the sowing of soybeans.

A study by Trezzi *et al.* (2015) indicated that *C. bonariensis* can reduce soybean yield by 50% at a plant density of just 2.7 plants m^{-2}. Therefore, the adoption of control measures -most of which involve chemical control- is essential to prevent losses in crop yield. The three species of *Conyza* have 105 reported cases of biotypes resistant to herbicides, such as glyphosate, paraquat, and ALS inhibitors (12). In Brazil, the three species have been reported to be glyphosate resistant, with *C. sumatrensis* having seven records of resistance to herbicides, the largest number accounted for in the country. Moreover, there are reports of multiple resistance to glyphosate and chlorimuron (28), to paraquat (1), and to 2,4-D (27), among others. In this sense, an initial application of systemic herbicides, with sequential application of burndown and/or pre-emergent herbicides has proved to be effective and necessary, especially for large plants with a history of herbicide resistance (8, 12). Herbicides such as PROTOX or ALS-inhibiting herbicides can be used in sequential applications (12, 26, 34).

Considering the aggressiveness of *C. sumatrensis* and the increasing number of reports of biotypes resistant to glyphosate and other herbicides, the need for proactive management is apparent. The combination and rotation of systemic herbicides, with pre-emergence and/or burndown action during sequential application, are effective in controlling this weed species. In this context, the objective of this study was to evaluate the efficacy of different herbicides by analyzing the sequential application of saflufenacil/imazethapyr in soybean pre-sowing.

Material and Methods

Experiments 1 and 2 of the study were conducted in the field during the 2018-2019 growing season in the municipalities of Assis Chateaubriand (24°28'28.0" S 53°51'39.8" W) and Alto Piquiri, state of Paraná (PR), Brazil (24°06'27.01"S 53°45'35.07" W), respectively. According to the Köppen classification, the climate of the region is Cfa, and the weather conditions during the experimental period are illustrated in figures 1 and 2.

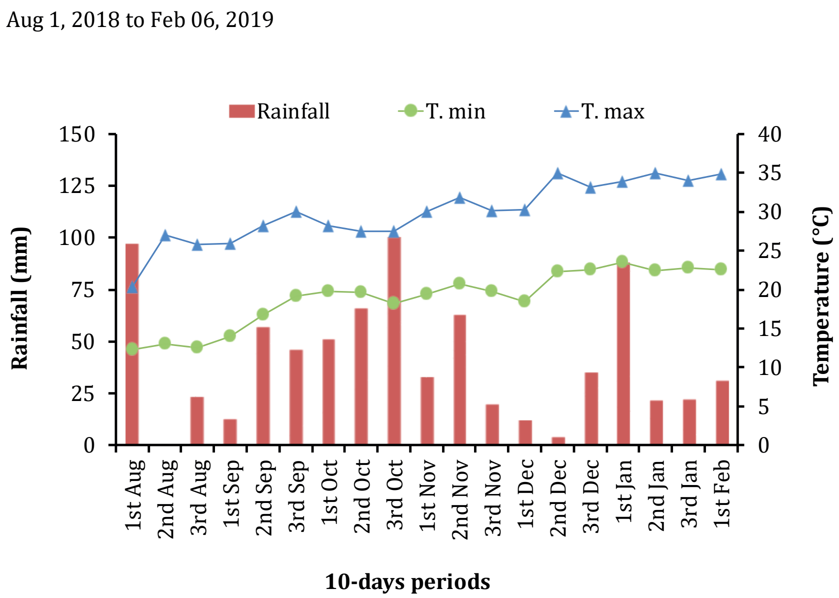

Figure 1. Rainfall and minimum and maximum temperatures for the experimental site Assis Chateaubriand (Experiment 1), 2018-19 growing season.

Figura 1. Representación de la lluvia, temperatura mínima y máxima para el sitio. Campaña 2018-19, Assis Chateaubriand (Experimento 1).

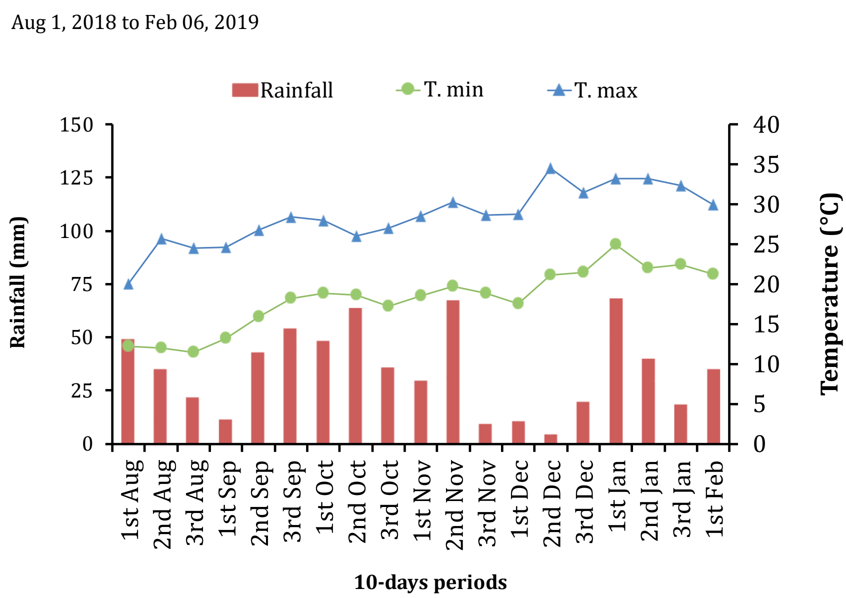

Figure 2. Representation of rainfall and minimum and maximum temperature for the test site Alto Piquiri (Experiment 2), 2018-19 growing season.

Figura 2. Representación de la lluvia, temperatura mínima y máxima para el sitio. Campaña 2018-19, Alto Piquiri (Experimento 2).

For experiment 1, the soil was classified as clayey (66.25% clay, 18.75% silt, and 15% sand), with the following chemical properties in the 0-20 cm layer: pH ($CaCl_2$) of 4.8, 2.23% organic matter (OM), and 11.85 $cmol_c$ dm^{-3} of cation exchange capacity (CEC). For experiment 2, the soil was classified as having a sandy loam texture (12.5% clay, 10% silt, and 77.5% sand), with a pH ($CaCl_2$) of 7.7, 1.39% OM, and 6.64 $cmol_c$ dm^{-3} of CEC.

Experimental site 1 was infested with *C. sumatrensis*, with a density of 6 plants m^{-2} (> 15 cm) and 11 plants m^{-2} (<15 cm), and approximately 55% of the *C. sumatrensis* population was resistant to paraquat. In experimental site 2, density was 8 plants m^{-2} (> 15 cm) and 2 plants m^{-2} (<15 cm), with approximately 10% of the *C. sumatrensis* population being resistant to paraquat. To determine the frequency of *C. sumatrensis* indicative of resistance to this herbicide, we applied paraquat (Gramoxone® 200) on tracks at label rate (400 g ai ha^{-1}) 14 days before the start of the experiment. Before and after application, the number of uncontrolled plants (≤20% control) with 6-10 leaves (4-8 cm in height) was evaluated and compared to the track where paraquat was not applied. Seven days after the application of paraquat, a second application was performed to determine the frequencies of resistance.

Previously, these areas had been cultivated with maize. Soybeans were sown under no-till, with 0.45 cm spacing between rows. Soybean cultivar Monsoy® 5917 IPRO (Monsanto Co. Brazil, São Paulo, SP, Brazil) was used in Experiment, 1 and Monsoy® 6410 IPRO (Monsanto Co. Brazil, São Paulo, SP, Brazil) in Experiment 2. The experiment was a randomized block design with four replicates. The treatments are presented in table 1. The dates of application, sowing, and environmental conditions during application are listed in table 2 (page 87). All applications were performed with a pressurized CO_2 backpack sprayer, equipped with six AIXR 110.015 nozzles, at a pressure of 2.5 kg * cm^{-2} and a speed of 3.6 km * h^{-1}, providing an application volume of 150 L * ha^{-1}.

Table 1. Treatments consisting of herbicide mixtures for controlling *Conyza sumatrensis* (2018-19 growing season).

Tabla 1. Tratamientos compuestos por mezclas de herbicidas para el control de *C. sumatrensis*. Campaña 2018-19.

	Treatments*	
	First application	**Sequential application**
1	Weedy control (no application)	No application
2	Glyphosate + 2,4-D + saflufenacil[1]	Paraquat[2]
3	Glyphosate + 2,4-D + saflufenacil[1]	Glyphosate + diclosulam
4	Glyphosate + 2,4-D + saflufenacil[1]	Flumioxazin/imazethapyr + paraquat[2]
5	Glyphosate + 2,4-D + saflufenacil[1]	Flumioxazin/imazethapyr[1]
6	Glyphosate + 2,4-D + saflufenacil[1]	Saflufenacil/imazethapyr + glyphosate[1]
7	Glyphosate + 2,4-D + saflufenacil[1]	Saflufenacil/imazethapyr + clethodim[1]
8	Glyphosate + 2,4-D + saflufenacil[1]	Glyphosate + imazethapyr[1]
9	Glyphosate + saflufenacil + diclosulam[1]	Saflufenacil/imazethapyr + glyphosate[1]
10	Glyphosate + diclosulam[1]	Saflufenacil/imazethapyr + glyphosate[1]
11	Glyphosate + 2,4-D[1]	Saflufenacil/imazethapyr + glyphosate[1]
12	Glyphosate + 2,4-D[1]	Glyphosate + diclosulam[1]

* Glyphosate (Zapp® QI 620 - 1,500 g ae ha^{-1}); 2,4-D (DMA® 806 BR - 670 g ae ha^{-1}); saflufenacil (Heat® - 35 g ai ha^{-1}); diclosulam (Spider® 840 WG - 25.2 g ai ha^{-1}); paraquat (Gramoxone® 200-400 g ai ha^{-1}); flumioxazin/imazethapyr (Zethamaxx® - 100/50 g ai/ae ha^{-1}); saflufenacil/imazethapyr (Optill® - 35.6/100.4 g ai/ae ha^{-1}); clethodim (Poquer® - 144 g ai ha^{-1}); imazethapyr (Vezir® 100-100 g ae ha^{-1}).

[1]Adjuvant Agral® (250 mL ha^{-1}) used. [2]Adjuvant Mees® (500 mL ha^{-1}) used.

* *glyphosate* (Zapp® QI 620 - 1,500 g ea ha^{-1}); 2,4-D (DMA® 806 BR - 670 g ea ha^{-1}); *saflufenacil* (Heat® - 35 g ia ha^{-1}); *diclosulam* (Spider® 840 WG - 25.2 g ia ha^{-1}); *paraquat* (Gramoxone® 200 - 400 g ia ha^{-1}); *flumioxazin/imazethapyr* (Zethamaxx® - 100/50 g ia/ea ha^{-1}); *saflufenacil/imazethapyr* (Optill® - 35.6/100.4 g ia/ea ha^{-1}); *clethodim* (Poquer® - 144 g ia ha^{-1}); *imazethapyr* (Vezir® 100 - 100 g ea ha^{-1}).

[1] Uso de adyuvante Agral® (250 mL ha^{-1}). [2] Uso de adyuvante Mees® (500 mL ha^{-1}).

Table 2. Dates and weather conditions during herbicide applications.
Tabla 2. Fechas y condiciones climáticas durante la aplicación de herbicidas.

	Date	Wind km h^{-1}	T. °C	Relative humidity %
Exp. 1 (first application)	Sep 10, 2018	6.2	24.2	57.0
Exp. 1 (sequential application)*	Sep 23, 2018	8.7	25.1	67.4
Exp. 2 (first application)	Sep 21, 2018	9.8	32.3	48.7
Exp. 2 (sequential application)*	Oct 4, 2018	2.8	31.2	58.9

* Day of sowing. / * Realizado el mismo día de la siembra.

The control of *C. sumatrensis* was evaluated at soybean sowing and 7, 21, and 35 days after the second application (DAA) of herbicides. Importantly, after the last control evaluation, all treatment plots were weeded except for the control. Injury to soybean plants was evaluated 14, 28, and 35 days after sowing (DAS), which was also performed in maize crops grown in succession areas. These evaluations were conducted through visual analysis at each experimental unit (0 for no injury, up to 100% for plant death), considering significantly visible symptoms in the plants according to their development (32).

Upon harvest, plant height and yield were evaluated. To evaluate the stand, the number of plants per meter was counted with four measurements per plot. Plant height was measured using a wooden ruler (10 plants per plot). For yield, the two central rows were harvested (4 m in length), moisture was corrected to 13%, and the results were extrapolated to kg ha^{-1}.

Analyses were performed using the statistical software Sisvar 5.6 (10). In addition, an analysis of variance (ANOVA) and an F-test ($P \leq 0.05$) were performed following Pimentel-Gomes and Garcia (2002), and mean treatment values were grouped using the Scott and Knott (1974) test ($P \leq 0.05$).

Results

For control evaluation at sowing, values of at least 91.5% were observed in Experiment 1 for the application of glyphosate + 2,4-D + saflufenacil and glyphosate + saflufenacil + diclosulam, The first evaluation was conducted 13 days after the first application, and the following evaluations included the effects of the two herbicides. In subsequent evaluations, all herbicide treatments resulted in the control of at least 90% *of C. sumatrensis*, with the exception of treatment 12. This treatment consisted of the first application of glyphosate + 2,4-D and the sequential application of glyphosate + diclosulam, and control at 35 DAA was only 56.8% (table 3, page 88).

The control results for *C. sumatrensis* in Experiment 2 were similar to those observed in Experiment 1. The highest percentages at soybean sowing were obtained for glyphosate + 2,4-D + saflufenacil and glyphosate + saflufenacil + diclosulam, with 75.3-87.5% of *C. sumatrensis* controlled. For the following evaluations, *C. sumatrensis* control of at least 80.3% (at 7 DAA) was recorded. At 35 DAA, with *C. sumatrensis* control between 93% and 99% in all evaluations, the only satisfactory control was found for treatment 12, which was 59.3% (table 4, page 88).

In Experiment 1, injury of up to 6% at 7 DAS was observed for treatments 3 (glyphosate + 2,4-D + saflufenacil sequential [*seq.*] glyphosate + diclosulam) and 12 (glyphosate + 2,4-D *seq*. glyphosate + diclosulam). However, a reduction in symptoms was observed in subsequent evaluations, even without any differences between treatments in the last evaluation at 35 DAS. Moreover, in the area of both experiments, maize was grown in the second crop (sowing in January 2019), and no crop injury was detected at 14, 28, and 35 DAS.

Table 3. *Conyza sumatrensis* control (%) after herbicide application. 2018-19 growing season, Assis Chateaubriand (Experiment 1).

Tabla 3. Control (%) de *C. sumatrensis* después de la aplicación de herbicidas. Campaña 2018-19, Assis Chateaubriand (Experimento 1).

Treatments			Sow	7 DAA	21 DAA	35 DAA
	First application	Sequential application	---------------%---------------			
1	Weedy control (no application)		0.0 d	0.0 d	0.0 c	0.0 c
2	Gly + 2,4-D + saflufenacil	Paraquat	91.5 a	92.0 b	93.3 a	92.5 a
3	Gly + 2,4-D + saflufenacil	Gly + diclosulam	92.3 a	93.0 b	97.3 a	96.8 a
4	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr + paraquat	93.5 a	94.8 a	95.3 a	94.8 a
5	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr	95.3 a	96.0 a	96.5 a	95.5 a
6	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + gly	94.0 a	98.3 a	98.8 a	99.8 a
7	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + clethodim	94.8 a	98.0 a	98.5 a	99.0 a
8	Gly + 2,4-D + saflufenacil	Gly + imazethapyr	92.0 a	95.3 a	96.8 a	94.8 a
9	Gly + saflufenacil + diclosulam	Saflufenacil/imazethapyr + gly	95.8 a	98.3 a	99.3 a	99.5 a
10	Gly + diclosulam	Saflufenacil/imazethapyr + gly	31.3 c	90.0 b	96.3 a	95.0 a
11	Gly + 2,4-D	Saflufenacil/imazethapyr + gly	44.0 b	92.0 b	97.3 a	96.8 a
12	Gly + 2,4-D	Gly + diclosulam	44.3 b	41.3 c	58.8 b	56.8 b
		Mean	72.2	82.3	85.6	85.1
		CV (%)	5.5	4.4	5.0	5.0
		F	*	*	*	*

Sow: sowing; DAA: day after sequential application; Gly: glyphosate. * Means followed by different letters in the same column are significantly different according to the Scott and Knott's (1974) test, $P \leq 0.05$.

Sow: siembra. DAA: día después de la aplicación secuencial. gly: *glyphosate*. * Las medias seguidas de la misma letra en la columna no difieren entre sí por Scott y Knott (1974), $P \leq 0,05$.

Table 4. *Conyza sumatrensis* control (%) after herbicide application. 2018-19 growing season, Alto Piquiri (Experiment 2).

Tabla 4. Control (%) de *C. sumatrensis* después de la aplicación de herbicidas. Campaña 2018-19, Alto Piquiri (Experimento 2).

Treatments			Sow	7 DAA	21 DAA	35 DAA
	First application	Sequential application	----------------%----------------			
1	Weedy control (without application)		0.0 d	0.0 d	0.0 d	0.0 c
2	Gly + 2,4-D + saflufenacil	Paraquat	75.8 b	83.5 b	85.0 b	96.5 a
3	Gly + 2,4-D + saflufenacil	Gly + diclosulam	76.8 b	80.3 b	83.8 b	96.3 a
4	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr + paraquat	83.0 a	90.8 a	93.3 a	96.5 a
5	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr	81.0 b	84.3 b	90.8 a	93.0 a
6	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + gly	86.0 a	91.3 a	96.3 a	99.3 a
7	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + clethodim	80.0 b	87.5 a	93.3 a	99.0 a
8	Gly + 2,4-D + saflufenacil	Gly + imazethapyr	75.3 b	81.0 b	87.5 b	94.3 a
9	Gly + saflufenacil + diclosulam	Saflufenacil/imazethapyr + gly	87.5 a	92.8 a	95.8 a	98.5 a
10	Gly + diclosulam	Saflufenacil/imazethapyr + gly	46.3 d	83.5 b	92.8 a	97.3 a
11	Gly + 2,4-D	Saflufenacil/imazethapyr + gly	57.5 c	82.0 b	89.5 b	96.5 a
12	Gly + 2,4-D	Gly + diclosulam	50.3 d	53.3 c	52.8 c	59.3 b
		Mean	66.6	75.8	80.0	85.5
		CV (%)	8.4	5.3	4.6	3.6
		F	*	*	*	*

Sow: sowing. DAA: day after sequential application. gly: glyphosate. * Means followed by different letters in the same column are significantly different according to the Scott and Knott's (1974) test, $P \leq 0.05$.

Sow: siembra. *DAA*: día después de la aplicación secuencial. gly: *glyphosate*. * Las medias seguidas de la misma letra en la columna no difieren entre sí por Scott y Knott (1974), $P \leq 0,05$.

In addition, no differences were found between treatments for plant height in Experiment 1. There was also a reduction in yield for the control, as well as for treatment 12, which resulted in the lowest control of *C. sumatrensis* and lowest soybean yield among all herbicide treatments. This may be because we used an earlier cycle cultivar and due to a water deficit in the first and second 10-day periods in December, with only 17 mm of rainfall and maximum average temperatures of 30.3°C and 35.1°C being observed during this time. For the control and treatment 12, in addition to weather conditions, competition with *C. sumatrensis* plants further reduced yield (table 5).

Table 5. Percentage of plant injury, height, and yield of soybean plants after herbicide application for control of *Conyza sumatrensis*. 2018-19 growing season, Assis Chateaubriand (Experiment 1).

Tabla 5. Daño al cultivo, altura y rendimiento de las plantas de soya después de la aplicación del herbicida, para el control de *C. sumatrensis*. Campaña 2018-19, Assis Chateaubriand (Experimento 1).

Treatments			Injury			H	Yield
			14 DAS	28 DAS	35 DAS		
	First application	Sequential application	----------%---------			cm	kg ha^{-1}
1	Weedy control (without application)		0.0 a	0.0 a	0.0	50.5	393 c
2	Gly + 2,4-D + saflufenacil	Paraquat	0.5 a	0.5 a	0.0	61.2	1,151 a
3	Gly + 2,4-D + saflufenacil	Gly + diclosulam	6.0 c	2.0 b	1.0	55.3	1,105 a
4	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr + paraquat	2.5 b	1.3 a	0.5	55.9	1,132 a
5	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr	2.5 b	1.0 a	0.8	56.9	1,071 a
6	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + gly	1.5 a	1.5 b	0.0	58.3	1,171 a
7	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + clethodim	2.5 b	1.8 b	0.5	59.9	1,126 a
8	Gly + 2,4-D + saflufenacil	Gly + imazethapyr	1.5 a	0.8 a	0.3	61.0	1,068 a
9	Gly + saflufenacil + diclosulam	Saflufenacil/imazethapyr + gly	3.3 b	2.0 b	0.8	59.8	1,095 a
10	Gly + diclosulam	Saflufenacil/imazethapyr + gly	3.5 b	1.3 a	0.8	57.3	1,081 a
11	Gly + 2,4-D	Saflufenacil/imazethapyr + gly	1.8 a	0.8 a	0.5	59.1	1,055 a
12	Gly + 2,4-D	Gly + diclosulam	6.0 c	2.8 b	1.3	60.0	789 b
		Mean	2.6	1.3	0.5	57.9	1,020
		CV (%)	14.5	19.3	19.5	6.6	12.2
		F	*	*	ns	ns	*

DAS: days after sowing; Gly: glyphosate; H: height. * Means followed by different letters in the same column are significantly different according to the Scott and Knott's (1974) test, $P \leq 0.05$. ns: non-significant, or means do not differ from each other according to the F-test ($P > 0.05$).

DAS: días después de la siembra. gly: *glyphosate*. *H:* altura. * Las medias seguidas de la misma letra en la columna no difieren entre sí por Scott y Knott (1974), $P \leq 0{,}05$. ns: no significativo, los medios no difieren entre sí por la prueba F ($P > 0{,}05$).

In Experiment 2, differences were detected between treatments in the three evaluations of injury to plants, with stronger symptoms (up to 5.3%) at 14 DAS and symptom reduction in the subsequent evaluations. At 35 DAS, the strongest symptoms were observed for treatments 3 and 12, as in Experiment 1, and for treatments 9 (glyphosate + saflufenacil + diclosulam *seq*. saflufenacil/imazethapyr + glyphosate) and 10 (glyphosate + diclosulam *seq*. saflufenacil/imazethapyr + glyphosate), with values of 3-3.5% injury. No differences were observed between treatments with respect to plant height. Differences in yield were observed, whereas in Experiment 1, reductions were observed in the control and treatment 12 due to competition with *C. sumatrensis* plants. The other herbicide treatments were superior to these two, but showed no significant differences from each other, with values of up to 4,104 kg ha^{-1} (table 6, page 90).

Table 6. Percentage of plant injury, height, and yield of soybean plants after herbicide application for the control of *Conyza sumatrensis*. 2018-19 growing season, Alto Piquiri (Experiment 2).

Tabla 6. Daño al cultivo, altura y rendimiento de las plantas de soja después de la aplicación del herbicida, para el control de *C. sumatrensis*. Campaña 2018-19, Alto Piquiri (Experimento 2).

Treatments			Injury			H	Yield
			14 DAS	28 DAS	35 DAS		
	First application	Sequential application	----------%----------			cm	kg ha^{-1}
1	Weedy control (without application)		0.0 a	0.0 a	0.0 a	66.3	2,567 b
2	Gly + 2,4-D + saflufenacil	Paraquat	0.0 a	0.0 a	0.0 a	83.1	3,924 a
3	Gly + 2,4-D + saflufenacil	Gly + diclosulam	5.3 d	3.5 c	3.0 b	77.8	4,076 a
4	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr + paraquat	1.3 b	0.3 a	0.0 a	75.4	3,944 a
5	Gly + 2,4-D + saflufenacil	Flumioxazin/imazethapyr	1.5 b	1.0 b	0.0 a	77.2	3,943 a
6	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + gly	2.5 c	1.8 b	0.8 a	75.7	3,906 a
7	Gly + 2,4-D + saflufenacil	Saflufenacil/imazethapyr + clethodim	2.8 c	1.8 b	0.8 a	75.1	3,994 a
8	Gly + 2,4-D + saflufenacil	Gly + imazethapyr	1.5 b	0.5 a	0.0 a	75.9	4,104 a
9	Gly + saflufenacil + diclosulam	Saflufenacil/imazethapyr + gly	5.0 d	3.8 c	3.0 b	74.1	4,067 a
10	Gly + diclosulam	Saflufenacil/imazethapyr + gly	4.5 d	3.8 c	3.0 b	73.1	4,035 a
11	Gly + 2,4-D	Saflufenacil/imazethapyr + gly	1.3 b	0.8 a	0.3 a	75.0	3,865 a
12	Gly + 2,4-D	Gly + diclosulam	5.3 d	4.0 c	3.5 b	73.7	3,101 b
		Mean	2.6	1.8	1.2	75.2	3,794
		CV (%)	8.6	13.0	11.2	6.4	9.7
		F	*	*	*	ns	*

DAS: days after sowing; Gly: glyphosate; H: height. * Means followed by different letters in the same column are significantly different according to the Scott and Knott's (1974) test, $P \leq 0.05$. ns: non-significant, or means do not differ from each other according to the F-test ($P > 0.05$).

DAS: días después de la siembra. gly: *glyphosate*. *H:* altura. * Las medias seguidas de la misma letra en la columna no difieren entre sí por Scott y Knott (1974), $P \leq 0,05$. ns: no significativo, los medios no difieren entre sí por la prueba F ($P > 0,05$).

Discussion

In both experiments, treatments with sequential application of saflufenacil/imazethapyr and flumioxazin/imazethapyr were among the most effective in controlling *C. sumatrensis*. According to Hedges *et al.* (2019), the pre-sowing application of dicamba/glyphosate (1,800 g ae ha^{-1}) + saflufenacil (25 g ai ha^{-1}) or saflufenacil/imazethapyr (100 g ae ha^{-1}) was effective in controlling *C. canadensis*, with results of ≥91% (12 weeks after application). Similarly, Cantu *et al.* (2021) reported the effectiveness of dicamba, in combination with other herbicides, in the control of *C. sumatrensis.* Moreover, Hedges *et al.* (2019) observed up to 10% soybean injury with the application of saflufenacil in different chemical management programs; however, the symptoms did not result in reduced soybean yield, thus demonstrating that the application of saflufenacil is effective in different management programs to control *Conyza* spp. (4, 7, 15, 16, 17, 34). Other studies also demonstrated, as in the present study, the efficacy of flumioxazin in different combinations for the control of *Conyza* spp. (20, 23, 26, 34).

In this study, the application of treatment 12 (glyphosate + 2,4-D *seq.* glyphosate + diclosulam) was not satisfactorily effective in controlling *C. sumatrensis* in both experiments; rather, it was the most phytotoxic treatment to soybean plants, with up to 6% at 14 DAA, as observed in Experiment 1. From a control point of view, an additional post-emergence application is necessary for soybean. Neto *et al.* (2009) observed an injury of 2.2% in soybean plants treated with glyphosate (960 g ae ha^{-1}) + diclosulam (35 g ai ha^{-1}) at the V1 stage. These symptoms did not affect soybean yield, indicating the selectivity of the combination.

Furthermore, soil and climate conditions interfere with the effects of diclosulam and other pre-emergents on soybean; for example, high rainfall after application can increase soybean injury (8), which may explain what was observed in the present study. According to Pereira *et al.* (2000) and Osipe *et al.* (2014), the application of diclosulam to soybean was selective, alone or in combination with glyphosate, although some symptoms of injury were observed.

The results presented herein demonstrate the importance of herbicide application before soybean sowing for the effective control of *C. sumatrensis*. The use of systemic herbicides in the first application, as well as the sequential application of burndown herbicides, is essential for the control of large plants (>15 cm height), notably saflufenacil and flumioxazin (PROTOX inhibitors), in addition to imazethapyr (ALS inhibitor) herbicides. These herbicides are also effective in controlling other weeds, especially broadleaf weeds such as *Amaranthus* spp., *Ipomoea* spp., and *Commelina* spp., and imazethapyr has been shown to act on *Digitaria insularis*, *D. horizontalis*, and other monocotyledons. Furthermore, our results indicate the effectiveness of the sequential application of these herbicides in controlling *C. sumatrensis*, which is relevant as they might contribute to supporting the ban on paraquat in Brazil.

Finally, control of *Conyza* spp. and other weeds cannot be left solely to glyphosate, as this genus has 66 cases of glyphosate-resistant biotypes worldwide. For instance, in Brazil, *Lolium perenne* ssp. *multiflorum*, *C. bonariensis, C. sumatrensis*, *C. canadensis*, *D. insularis*, *Chloris elata*, *Amaranthus palmeri*, *Eleusine indica*, and *Amaranthus hybridus* have glyphosate-resistant biotypes (12). Thus, the use of other herbicides, such as ALS inhibitors, auxinics, and glufosinate, should be considered. This is especially true for combinations and weed management during the off-season, as studies have highlighted the efficacy of pre-emergence herbicides along with pre-sowing burndown for weed management in grain crops (2, 3, 5, 21, 31).

Conclusion

Control levels were high for all the herbicide treatments, except for the one that was free of saflufenacil in both applications. These results highlight the importance of saflufenacil in controlling *C. sumatrensis* and show promise for saflufenacil/imazethapyr considering the system and other weeds. Finally, all studied treatments were selective to soybean, which showed higher injury values in the presence of diclosulam; however, this did not compromise soybean agronomic performance.

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Interference and threshold level of *Sida rhombifolia* in transgenic soybean cultivars

Interferencia y nivel de daño económico de *Sida rhombifolia* en cultivares de soja transgénica

Leandro Galon [1*], Alessandro Konzen [1], Maico André Michelon Bagnara [1], Leonardo Brunetto [1], Ignacio Aspiazú [2], Antônio Marcos Loureiro da Silva [1], Daiane Brandler [3], Hugo Von Linsingen Piazzetta [1], André Luiz Radünz [4], Gismael Francisco Perin [1]

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Abstract

This study aimed to assess the interference and threshold level (TL) of *Sida rhombifolia,* the arrowleaf sida, competing with different soybean cultivars. The treatments comprised different soybean cultivars (NS 6909, NA 5909, DM 5958, Brasmax ELITE, Brasmax LANÇA, and SYN 13561) and densities of arrowleaf sida plants per square meter (m^{-2}) (0, 2, 3, 4, 9, 15, 16, 23, 22, and 58; 0, 2, 3, 3, 6, 6, 10, 11, 18, and 47; 0, 3, 4, 7, 8, 10, 11, 13, 15, and 24; 0, 1, 4, 6, 12, 18, 19, 31, 44, and 50; 0, 4, 5, 6, 9, 13, 17, 20, 20, and 47; 0, 2, 3, 5, 9, 11, 15, 18, 29, and 30, respectively) for each cultivar. Cultivars NS 6909, NA 5909, and Brasmax Lança were more competitive than DM 5958, Brasmax Elite, and SYN 13561. The TL values varied from 0.55 to 0.95 plants m^{-2} for cultivars NS 6909, NA 5909, and Brasmax Lança, which exhibited greater competitiveness with arrowleaf sida. The lowest values of TL varied from 0.26 to 0.61 plants m^{-2} for cultivars DM 5958, Brasmax Elite, and SYN 13561, which had less competitiveness with weed.

Keywords
Glycine max • arrowleaf sida • integrated weed management

1 Federal University of Fronteira Sul. Campus Erechim. Laboratory of Sustainable Management of Agricultural Systems. 99700-970. Erechim. Rio Grande do Sul. Brazil. * leandro.galon@uffs.edu.br
2 State University of Montes Claros. Department of Agricultural Sciences. 39440-000. Janaúba. Minas Gerais. Brazil.
3 Federal University of Technology Paraná. Campus Pato Branco. Laboratory of Weed Science Research Centre. 85503- 390. Pato Branco. Paraná. Brazil.
4 Federal University of Fronteira Sul. Campus Chapecó. Department of Agronomy. 89815-899. Chapecó. Santa Catarina. Brazil.

Resumen

El objetivo de este estudio fue evaluar la interferencia y determinar el nivel de daño económico (NDE) de escoba dura infestando diferentes cultivares de soja. Los tratamientos fueron cultivares de soja (NS 6909, NA 5909, DM 5958, Brasmax ELITE, Brasmax LANÇA y SYN 13561) y las densidades de escoba dura (0, 2, 3, 4, 9, 15, 16, 23, 22 y 58; 0, 2, 3, 3, 6, 6, 10, 11, 18 y 47; 0, 3, 4, 7, 8, 10, 11, 13, 15 y 24; 0, 1, 4, 6, 12, 18, 19, 31, 44 y 50; 0, 4, 5, 6, 9, 13, 17, 20, 20 y 47; e 0, 2, 3, 5, 9, 11, 15, 18, 29 y 30 plantas m^{-2}), respectivamente, para cada cultivar. Los cultivares NS 6909, NA 5909 y Brasmax Lança son más competitivos que DM 5958, Brasmax Elite y SYN 13561. Los valores de NDE varían de 0,55 a 0,95 plantas m^{-2} para los cultivares NS 6909, NA 5909 y Brasmax Lança, que mostraron mejor competitividad. Los valores más bajos de NDE varían de 0,26 a 0,61 plantas m^{-2} para los cultivares DM 5958, Brasmax Elite y SYN 13561, los cuales mostraron menor competitividad con la maleza.

Palabras clave
Glycine max • escoba dura • manejo integrado de malezas

Introduction

Knowledge and management of factors that lead to reduced crop yield is essential for farmers to obtain better productive results considering the importance of soybeans in Brazil. Weeds can cause significant losses if not properly managed. Competition losses can vary from 2-94% depending on factors such as soybean cultivars, weed densities and species, emergence times, and edaphoclimatic conditions (14, 19, 29, 32).

In general, weeds compete with crops for environmental resources such as water, light, and nutrients and can cause high losses in soybean yield when left uncontrolled (13, 14, 17 22, 23, 32). Among the weeds that cause losses in soybean crops, *Sida rhombifolia*, the arrowleaf sida, stands out mainly because of its adaptability to low fertility, acidic soils, and high competitive ability (12). In addition, it has amphistomatic leaves with anomocytic stomata that can readily adapt to the environment where they grow and develop (11). Therefore, the correct management of arrowleaf sida is essential in soybean crops because its occurrence causes direct (such as reduced grain yield) and indirect losses (such as virus transmission to many crops by being the hosts of silverleaf whitefly) (23).

According to Agostinetto *et al*. (2010) and Zandoná *et al*. (2018), in addition to understanding the damage caused by competition, it is necessary to understand the influence of weed density and distribution when they coexist with crops in a given field. Owing to the efficiency, practicality, and low cost of herbicides, these are used as general management strategies for weeds compared with other control methods (31). Despite the advantages of chemical control, the search for more sustainable production models is a priority in the current discussions on grain production based on threshold level (TL) strategies for weed management. Thus, TL is an essential tool for farmers, allowing crop monitoring and decision making about the most appropriate time and way to manage weeds.

The TL concept recommends that applying herbicides or other control methods is justified only when the damage caused by weeds is more than the cost of control measures (1, 21). High densities of weeds competing with crops render decision-making by producers easier as adopting control measures with low population densities of weeds becomes difficult owing to the quantification of economic advantages associated with the control costs (21).

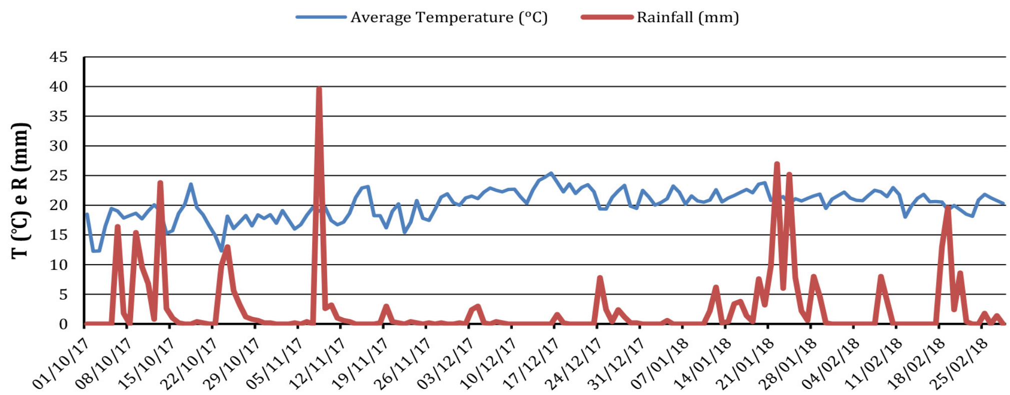
Mathematical models have been used to estimate the loss of grain yield owing to the presence of weeds (18). The hyperbolic relationship between grain yield and weed population was first described by Cousens (1985), wherein an empirical rectangular hyperbola model was adjusted to predict yield loss as a function of weed population demonstrating its superiority over the other models. The rectangular hyperbola model is based on the non-linear relationship between the percentage of yield loss due to interference in competition-free control and weed population (10). It incorporates parameter *i,* which represents the loss of production caused by adding the first weed, and parameter *a*, which symbolizes the loss of crop production as weed density approaches infinity. The biological significance of the model demonstrates that the competition effect of each weed added to the crop decreases as weed density increases owing to intraspecific competition. Initially, yield loss is proportional, but the loss decreases above a particular weed density.

We hypothesized that there would be differences in the competitive abilities of the crop and TL because of soybean cultivars coexisting with increasing densities of arrowleaf sida. Therefore, the objective of this study was to evaluate the interference and TL of arrowleaf sida competing with different soybean cultivars.

Material and methods

Site

The experiment was conducted under field conditions in an experimental area of the Federal University of Fronteira Sul, Campus Erechim/RS/BR, with geographic coordinates of 27°43'47" S and 52°17'37" W, from October 2017 to February 2018. The soil is classified as humic aluminoferric red latosol at an altitude of 670 m above sea level (15). The local climate is Cfa, i.e., a humid temperate climate having hot summers, wherein rains are distributed well throughout the year, based on the classification established by Köppen (figure 1) (8, 20).



Source: Inmet (2018).
Fuente: Inmet (2018).

Figure 1. Rainfall and average daily temperature during the soybean crop cycle from October 2017 to February 2018, UFFS, Erechim/RS.

Figura 1. Precipitación y temperatura media diaria durante el ciclo del cultivo de la soja de octubre de 2017 a febrero de 2018, UFFS, Erechim / RS.

The pH correction and fertilization in the soil were performed based on physicochemical analysis following the technical recommendations for soybean crops (26). The physicochemical characteristics of the soil were: pH = 5.1; Organic matter = 3.0%; P = 5.2 mg dm^{-3}; K = 118 mg dm^{-3}; Al^{3+} = 0.3 $cmol_c$ dm^{-3}; Ca^{2+} = 5.5 $cmol_c$ dm^{-3}; Mg^{2+} = 3.0 $cmol_c$ dm^{-3}; cation exchange capacity (CEC) = 7.4 $cmol_c$ dm^{-3}; CEC at pH_7= 16.6 $cmol_c$ dm^{-3}; H + Al = 7.7 $cmol_c$ dm^{-3}; sum of bases = 53%, and clay content = 60%. The no-tillage system was employed, and vegetation was dried using glyphosate herbicide at a concentration of 1440 g ae ha^{-1}, 20 d before sowing soybean cultivars with a sowing-fertilizing machine on October 4th 2017, distributing 480 kg ha^{-1} based on the formula NPK 02-20-20 in sowing furrow.

Experimental design

The experimental design was completely randomized, with four replicates, and the treatments were composed of six soybean cultivars, i.e., NS 6909 IPRO, NA 5909 RG IPRO, DM 5958 RSF, Brasmax Elite IPRO, Brasmax Lança IPRO, and SYN 13561 IPRO, and ten densities of arrowleaf sida for each cultivar (0, 2, 3, 4, 9, 15, 16, 23, 22, and 58 plants m^{-2}; 0, 2, 3, 3, 6, 6, 10, 11, 18, and 47 plants m^{-2}; 0, 3, 4, 7, 8, 10, 11, 13, 15, and 24 plants m^{-2}; 0, 1, 4, 6, 12, 18, 19, 31, 44, and 50 plants m^{-2}; 0, 4, 5, 6, 9, 13, 17, 20, 20, and 47 plants m^{-2}; and

0, 2, 3, 5, 9, 11, 15, 18, 29, and 30 plants m^{-2}). As the arrowleaf sida weed originated from the soil seed bank, the establishment of densities varied with factors such as competition for resources, vigor, and humidity, which prevented the exact number of plants per area of experimental unit from being established. The different densities of arrowleaf sida acted as replicates, providing the necessary variation for the statistical analysis performed using the nonlinear model proposed by Cousens (1985) and Agostinetto *et al*. (2010).

Plot management

Each experimental unit consisted of a 15 m^2 area (5 m × 3 m) having six soybean lines spaced 0.50 m apart with four central lines considered appropriate evaluation areas and discounting 1 m of front and end borders of each plot. The sowing density of different soybean cultivars was 14 viable seeds per meter, corresponding to a density of 280,000 seeds ha^{-1}, i.e., 28 plants m^{-2}. Soybean cultivars were selected based on their characteristics of undetermined growth and genetic differences. These are also the most cultivated soybean varieties in Rio Grande do Sul. All six cultivars bear resistance to insects and tolerance to herbicides but differ in crop cycles and maturation groups. NA 5909 RG IPRO and Brasmax Elite IPRO have early crop cycles, SYN 13561 IPRO has an early to medium cycle, and NS 6909 IPRO, DM 5958 RSF IPRO, and Brasmax Lança IPRO possess medium cycles. In addition, the cultivars NA 5909 RG IPRO, Brasmax Elite IPRO, SYN 13561 IPRO, NS 6909 IPRO, DM 5958 RSF IPRO, and Brasmax Lança IPRO belong to maturation groups 6.2, 5.5, 6.1, 6.3, 5.8, and 5.8, respectively.

We applied glyphosate herbicide at a concentration of 1440 g ha^{-1} to the soil to reduce the density of competing weed species as the crop reached V3 to V4 phenological stages 40 d after emergence (DAE), and the weed was at the two-four leaves stage. We selected the season because of its suitability for applying herbicides post the emergence of genetically modified soybeans. The arrowleaf plants were protected using plastic cups and buckets to prevent herbicide damage. The application was performed using a precision CO_2-pressurized backpack sprayer equipped with four DG 110.02 spray tips, maintaining a constant pressure of 210 kPa and a travel speed of 3.6 km ha^{-1}, which provided a herbicide spray flow of 150 L ha^{-1}.

Evaluated variables and sampling

The quantification of the plant density (PD), leaf area (LA), soil cover (SC), and dry mass (DM) of the shoots of arrowleaf sida was performed 54 d after crop emergence.The number of plants present within two plots with an area of 0.25 m^2 (0.5 m × 0.5 m) per plot was counted to determine PD. SC was evaluated visually by two individual evaluators, using a percentage scale on which a score of zero corresponded to the absence of cover, and a score of 100 represented the total SC. A portable LA meter model, CI-203 BioScence, was used to determine the LA (cm^2 m^{-2}) by collecting the plants in the center of each experimental unit in an area of 0.25 m^2 (0.5 m × 0.5 m). To determine DM after measuring LA, arrowleaf sida plants were placed in kraft paper bags and dried in a forced-air circulation oven at 72°C until no further weight change occurred.

At the end of the soybean cycle, the grain yields of cultivars were determined by harvesting plants in an area of 6 m^2 (3 m × 2 m) for each experimental unit as the moisture content of grains reached approximately 15%. We determined the moisture content of grains by weighing them and correcting the obtained grain mass for a moisture content of 13%, which were then extrapolated for kg ha^{-1}. The soybean cultivars were harvested 130 d after sowing.

The percentage losses in the grain yield of soybean cultivars concerning experimental units free of competing plants were calculated using Equation 1:

$$\text{Loss (\%)} = \left(\frac{Ra - Rb}{Ra} \right) x\,100 \tag{1}$$

where

Ra and Rb = the crop yields with and without the presence of the competing arrowleaf sida plants, respectively.

The values of DM (g m^{-2}), SC (%), and LA (cm^2) were multiplied by 100 before data analysis to eliminate the use of correction factors in the model (1, 29).

Statistical analysis

We determined the association between the percentages of grain yield loss and explanatory variables separately for each soybean cultivar using a nonlinear regression model derived from rectangular hyperbola proposed by Cousens (1985) according to Equation 2:

$$\mathrm{YL} = \frac{(i * X)}{(1 + (\frac{i}{a}) * X)} \tag{2}$$

where

YL = yield loss (%)

X = PD, LA, SC and DM of arrowleaf sida.

i and a = the yield losses (%) per unit of the arrowleaf plant as the values of the variables approach zero and infinity, respectively.

Data adjustment for PD, SC, LA, and DM variables was performed using the Proc Nlin procedure of SAS to estimate the competitive ability and TL of the species using the mathematical modeling procedure (27). We used the Gauss-Newton method for estimating the values of parameters wherein the sum of the squares of deviations in observations for the adjusted values is minimized by successive iterations (1). The *F*-statistic value ($p \leq 0.05$) was used as the criterion for fitting the model to the data. The acceptance criterion for matching the model to the data was based on the highest coefficients of determination (R^2) and *F* values, and the lowest mean of squared residuals (MSR).

Parameter estimates obtained from the equation proposed by Cousens (1985) and adapted from Lindquist and Kropff (1996) were used to calculate the level of economic damage, i.e., TL (Equation 3).

$$\mathrm{TL} = \frac{(Cc)}{(R * P * (\frac{i}{100}) * (\frac{H}{100}))} \tag{3}$$

where

TL = the threshold level (plants m^{-2})

Cc = the control cost of herbicide and tractor application (dollars ha^{-1})

R = the grain yield of soybean cultivars (kg ha^{-1})

P = soybean price (dollars kg^{-1} of grains)

i = yield loss (%) of soybean per unit of the competing plant as population level approaches zero

H = herbicide efficiency (%).

We estimated three values for variables Cc, R, P, and H (Equation 3). For Cc, we considered the average cost of tractor application, and the maximum and minimum costs were changed by 25% of this average cost. R was estimated based on the lowest, highest, and average yields obtained in Rio Grande do Sul over the last ten years (9). The soybean price P was estimated based on the lowest, highest, and average cost of soybeans paid per 60 kg bag in the last ten years (7). The values of H were established as 80, 90, and 100% of control, with 80% being the minimum weed control considered effective (27). For TL simulations, intermediate values were used for the uncalculated variables.

Results and discussion

Competitive ability of soybean cultivars with arrowleaf sida

The explanatory variables PD, LA, SC, and shoot DM of arrowleaf sida for all soybean cultivars exhibited significant *F* values (table 1).

The rectangular hyperbola model showed adjustments for all evaluated cultivars, with R^2 higher than 0.57 and low MSR values. Variations in data adjustment were observed in relation to the cultivar and studied variables, corroborated by results observed in literature for the rice competing with barnyardgrass (1), beans competing with alexandergrass (21), and soybeans competing with alexandergrass (19). Cargnelutti Filho and Storck (2007) considered the values of R^2 between 0.57 to 0.66 as moderate to good when working with the genetic variation, effect of cultivars, and heritability of corn hybrids, which partly agree with the results observed in this study.

Table 1. Rectangular hyperbola model adjustments obtained for loss of grain yield, as a function of plant density, soil cover, leaf area, and dry mass arrowleaf sida (*Sida rhombifolia*) and soybeans cultivars.

Tabla 1. Ajustes del modelo de hipérbola rectangular obtenidos por pérdida de rendimiento de granos, en función de la densidad de plantación, la cobertura del suelo, el área foliar y la masa seca de los cultivares de afata (*Sida rhombifolia*) y cultivares de soja.

Relative explanatory variables	Parameters[1]		R^2	MSR	F
	i	a			
Density of arrowleaf sida plants					
NS 6909 IPRO	2.74	54.62	0.60	40.13	54.84*
NA 5909 RG IPRO	2.74	47.43	0.69	144.80	9.55*
DM 5958 RSF	4.36	49.40	0.85	19.37	120.34*
Brasmax Elite IPRO	2.41	32.76	0.62	20.86	69.25*
Brasmax Lança IPRO	3.77	64.44	0.89	14.91	249.72*
SYN 13561 IPRO	5.35	48.57	0.84	31.73	99.74*
Soil cover from arrowleaf sida plants					
NS 6909 IPRO	1.14	81.70	0.60	40.13	54.84*
NA 5909 RG IPRO	0.01	41.43	0.84	24.50	22.29*
DM 5958 RSF	0.04	43.39	0.78	24.85	115.98*
Brasmax Elite IPRO	2.14	43.87	0.69	9.83	148.14*
Brasmax Lança IPRO	0.08	37.93	0.71	79.27	42.37*
SYN 13561 IPRO	0.04	49.27	0.70	39.30	79.76*
Leaf area of arrowleaf sida plants					
NS 6909 IPRO	0.12	54.10	0.83	26.37	77.24*
NA 5909 RG IPRO	0.22	42.49	0.90	75.93	23.20*
DM 5958 RSF	0.005	32.41	0.73	34.59	65.65*
Brasmax Elite IPRO	0.003	28.41	0.57	25.85	55.36*
Brasmax Lança IPRO	0.009	34.59	0.60	82.73	41.72*
SYN 13561 IPRO	0.005	46.34	0.70	46.34	80.94*
Dry mass of the aerial part of arrowleaf sida plants					
NS 6909 IPRO	0.21	25.98	0.94	95.96	19.72*
NA 5909 RG IPRO	0.04	40.37	0.78	76.03	21.80*
DM 5958 RSF	0.32	28.87	0.74	31.21	73.17*
Brasmax Elite IPRO	0.23	23.31	0.68	25.66	60.69*
Brasmax Lança IPRO	0.10	44.00	0.77	40.41	89.61*
SYN 13561 IPRO	0.32	33.60	0.61	49.04	63.13*

[1] *i* and *a*: losses in productivity (%) per arrowleaf sida unity when the value of the variable approaches zero and infinity, respectively, obtained by the rectangular hyperbolic model Y= (*i*.X)/(1+(*i*/*a*). X (Cousens, 1985). *: Significant at $p \leq 0.05$. R^2: Determination coeficient. MSR: average square of residue.

[1] i y a: pérdidas de productividad (%) por unidad de afata cuando el valor de la variable se aproxima a cero o tiende a infinito, obtenido por el modelo de hipérbola rectangular Y= (i.X)/(1+(i/a).X (Cousens, 1985); respectivamente * Significativo a $p \leq 0{,}05$ R^2: Coeficiente de determinación MSR: Cuadrado medio del residuo.

The results revealed that the estimated values for parameter *i* tended to be higher for soybean cultivars DM 5958 RSF, Brasmax Elite IPRO, and SYN 13561 IPRO compared with the average values of all evaluated variables, i.e., PD, SC, LA, and DM (table 1, page 99). Additionally, the cultivars NS 6909 IPRO, NA 5909 RG IPRO, and Brasmax Lança IPRO were verified to be highly competitive, presenting lower grain yield losses compared to other cultivars, with lower average values of *i*. These competitive differences may be related to genetic differences present in the cultivars determining characteristics such as crop cycle, maturation group, height, LA index, root system, and ramifications through which plants defend themselves during a shortage of resources, i.e., light, water, and nutrients in the environment. These results are consistent with those reported by Butts *et al*. (2018), who evaluated the competition of *Amaranthus tuberculatus* with soybeans, and observed a 35% reduction in shoot biomass when the crop was put into competition with different densities of three biotypes of the weed species. The study also reported the number of arrowleaf sida required to cause more than 20% losses in soybean grain yield. We discovered that the loss was already evident in seven plants m^{-2} for cultivar SYN 13561 IPRO (21.15%) and in eight plants m^{-2} for DM 5958 RSF IPRO (20.44%) and Brasmax Lança IPRO (20.54%).

The other cultivars tested in the presence of these numbers per square meter exhibited below 18.7% loss in yield (table 1, page 99). Similarly, Galon *et al*. (2019) assessed soybean yield loss demonstrating the differential tolerance of the crop as the cultivars SYN 1059 IPRO, BMX Elite IPRO, and NS 5445 IPRO lost a lower percentage of grains than the cultivars NS 5959 RG IPRO and SYN 13561 IPRO when competing with different alexandergrass densities. Thus, the specific capacity of cultivars to coexist and tolerate the presence of various weed species, i.e., arrowleaf sida or alexandergrass, is evident based on the intrinsic characteristics of each cultivar.

The results revealed that as arrowleaf sida doubled the size of its LA, increasing from 250 cm^2 m^{-2} to 500 cm^2 m^{-2}, the cultivars NS 6909 IPRO, NA 5909 RG IPRO, DM 5958 RSF, Brasmax Elite IPRO, Brasmax Lança IPRO, and SYN 13561 IPRO lost 32.3%, 21.8%, 48.3%, 50.0%, 46.9%, and 48.7% of grain yield, respectively (table 1, page 99). because the loss in grain yield resulted from the failure of soybean to fully shade the soil until 54 DAE, allowing more light penetration through the community canopy, rendering the crop less competitive than arrowleaf sida plants.

When a crop is shaded, competition for solar radiation increases, making the resource search less efficient. Consequently, it is less able to develop and grow, thus decreasing grain yield (25). Similar results were observed by Galon *et al*. (2019), who tested different soybean cultivars in competition with alexandergrass densities and found that increases in the LA of the weed increased yield loss. The yield loss results of the soybean cultivars in relation to the SC percentage were similar to those observed for PD and LA, that is, an increase in the SC percentage of the arrowleaf plants increased crop damage (table 1, page 99). All soybean cultivars exhibited a high percentage reduction in grain yield with increased weed SC. This is consistent with the explanation for PD and LA because the plant that exhibits higher values obtains the advantage of competition over its neighbor, primarily due to light resources. Consequently, it shows more intense growth and development, as previously discussed.

Studies on *Digitaria ciliaris*, *Echinochloa crus-galli* var. crus-galli, *Bidens pilosa*, *Euphorbia heterophylla*, *Urochloa plantaginea*, and *Sida rhombifolia* demonstrate the high competitiveness of these species with soybeans (2, 4, 19, 21, 25). The incidence of sunlight owing to low SC can stimulate the emergence of weeds. However, there was no increase in germination in the presence of light for arrowleaf sida because it is insensitive to this condition (16), which allows the species to establish under a wide range of environmental conditions, and thus compete with the crops.

Accumulating 100 g m^{-2} of DM, arrowleaf sida decreased the grain yield of cultivars NS 6909 IPRO, DM 5958 RSF, Brasmax Elite IPRO, and SYN 13561 IPRO by more than 11%, and that of NA 5909 RG IPRO and Brasmax Lança IPRO by less than 4%. (table 1, page 99). Additionally, the same authors mentioned that cultivars with high stature, rapid emergence, and greater accumulation of biomass in shoots are more competitive. Bean cultivars competing with alexandergrass (21) exhibited different competitive behaviors, which are probably related to their different intrinsic characteristics, such as growth habit, development cycle, number of branches, and volume of the root system, which affect the competitive ability of the crop and cause differentiation between the cultivars competing with weeds.

The parameter *i* is an index used to compare the relative competitiveness of species (1). Different *i* values were observed for the soybean cultivars for the tested explanatory variables (table 1, page 99). Other studies have also compared the competitiveness of corn cultivars (3), soybeans (18), rice (18), beans (21), and wheat (30). The comparison between the soybean cultivars in terms of explanatory variables (PD, LA, SC, and DM) showed that the order of competitiveness was NA 5909 RG IPRO > NS 6909 IPRO > Brasmax Lança IPRO > DM 5958 RSF > Brasmax Elite IPRO > SYN 13561 IPRO (table 1, page 99). The differences between the results are primarily due to different genetic characteristics or the occurrence of a high standard error in the estimation of *i*, which can be attributed to variability associated with field experimentation (1). Other studies reported similar results when verifying that rice cultivars competing with rice grass (1), beans (21), or soy (19) responded differently in terms of the evaluated parameters when infested with weeds.

For all explanatory variables, cultivars from the same growth cycle or maturation group had different i-values (table 1, page 99). This demonstrates that soybean cultivars respond differently to competition with arrowleaf sida, primarily because of the morphophysiological characteristics of the cultivars, which define their ability to compete with weeds for environmental resources (3). Another explanation is related to the different statures of the cultivars, important in the competition for light, affecting yield, depending on the stage at which shading occurs in relation to the definition of the yield components (25). According to these authors, shaded leaves receive less intense and reflected light, which causes a gradual decrease in photosynthetic rate as they approach the ground.

The estimates of *a*, regardless of the explanatory variable, were all less than 100% (table 1, page 99), demonstrating that it was possible to adequately simulate the maximum losses in soybean grain yield with different densities of arrowleaf sida. If crops have high productive potential and adequate conditions for soil fertility, water availability, and luminosity, a lower daily percentage loss will be caused by certain species of weeds (21).

The comparison between the evaluated explanatory variables for all soybean cultivars demonstrated a better fit of the model in the order PD > LA > SC > DM, considering the highest mean values of R^2 and F and the lowest mean values of MSR (table 1, page 99), indicating that PD can be used to replace the other variables to estimate soybean grain yield losses.

To simulate the TL values, the PD of the arrowleaf sida was used, as it exhibited the best fit for the rectangular hyperbola model. It is the most commonly used variable in experiments with this objective owing to its ease, speed, and low cost (1, 19, 21).

Economic damage level of arrowleaf sida in soybean

The successful implementation of management systems for arrowleaf sida in soybean fields can be achieved by determining the density that exceeds TL. We observed that the cultivars NS 6909, NA 5909 RG IPRO, and Brasmax Elite IPRO had the highest TL values in all performed simulations, ranging 0.50-0.95 plants m^{-2} (figure 2, page 102 and figure 3, page 103).

The lowest TL values were obtained for cultivars DM 5958 RSF, Brasmax Elite IPRO, and SYN 13561 IPRO, ranging 0.26-0.61 plants m^{-2}. This is probably due to the lower initial growth speed or because they are very productive cultivars, and thus, are more sensitive to competition with weeds, even at low densities. According to Balbinot Jr. and Fleck (2005), cultivars that present a high growth rate at the beginning of the cycle and appropriate plant characteristics, especially stature, suffer less competition with weeds. Galon *et al.* (2019) also observed that soybean cultivars that presented the highest grain yields suffered the most competition with alexandergrass, that is, the lowest number of plants m^{-2} was necessary to reach the TL.

In the average of all soybean cultivars, there was a difference in TL of approximately 24% when comparing the lowest with the highest grain yields (figure 2A, page 102). Therefore, the higher the productive potential of the cultivars, the lower the density of arrowleaf plants required to overcome TL, making the adoption of control measures worthwhile. When evaluating the TL for alexandergrass infesting bean (21) and soybean (19) cultivars, it was observed that it varied depending on the evaluated cultivars, and those with the greatest productive potential demonstrated a smaller TL.

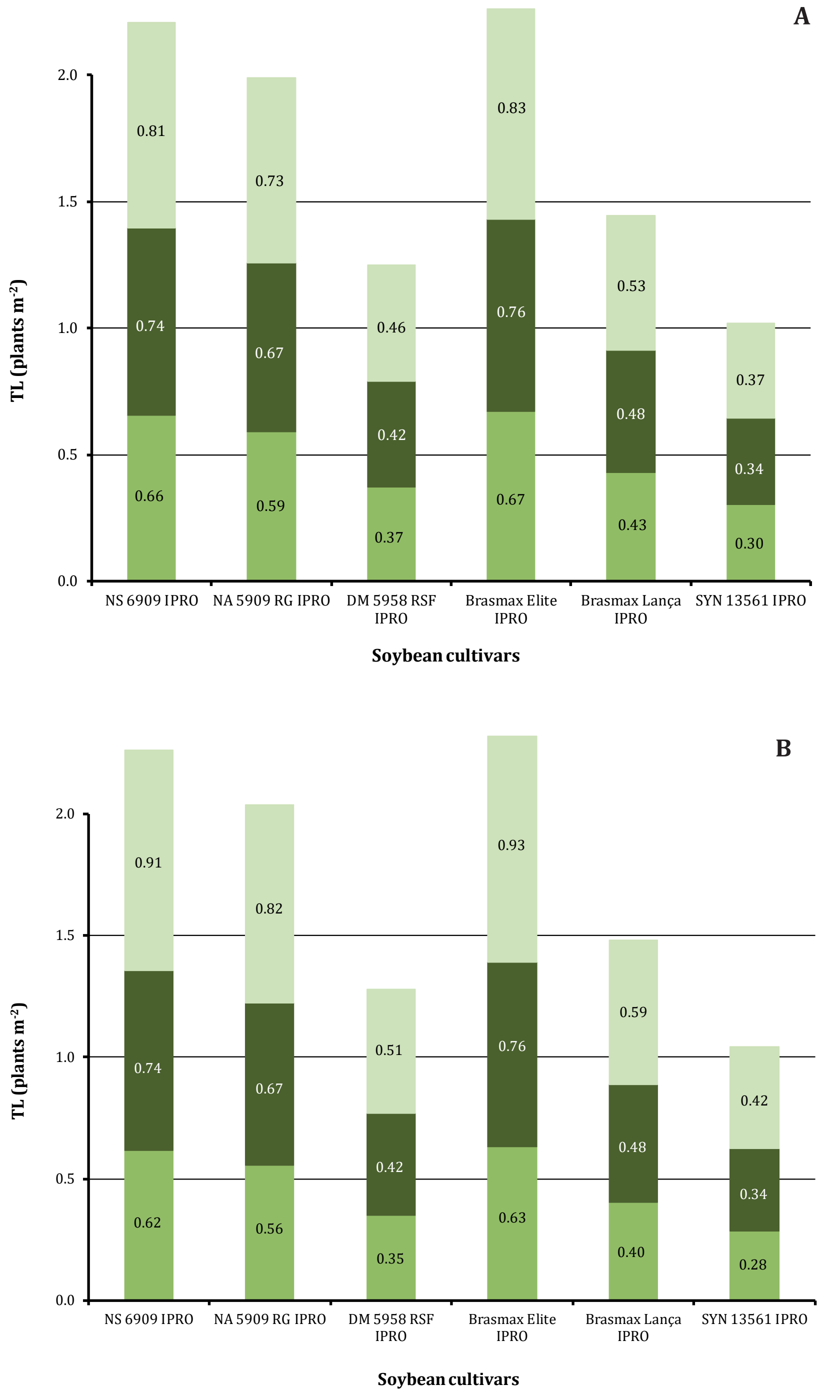


Figure 2. Threshold level (TL) of arrowleaf sida (*Sida rhombifolia*) as a function of soybean grain yield (A) and price of cultivars (B). UFFS, Erechim-RS, 2018.

Figura 2. Nivel de daño económico (TL) de escoba dura (*Sida rhombifolia*) en función del rendimiento de granos (A) y del precio (B) de soja cultivares. UFFS, Erechim-RS, 2018.

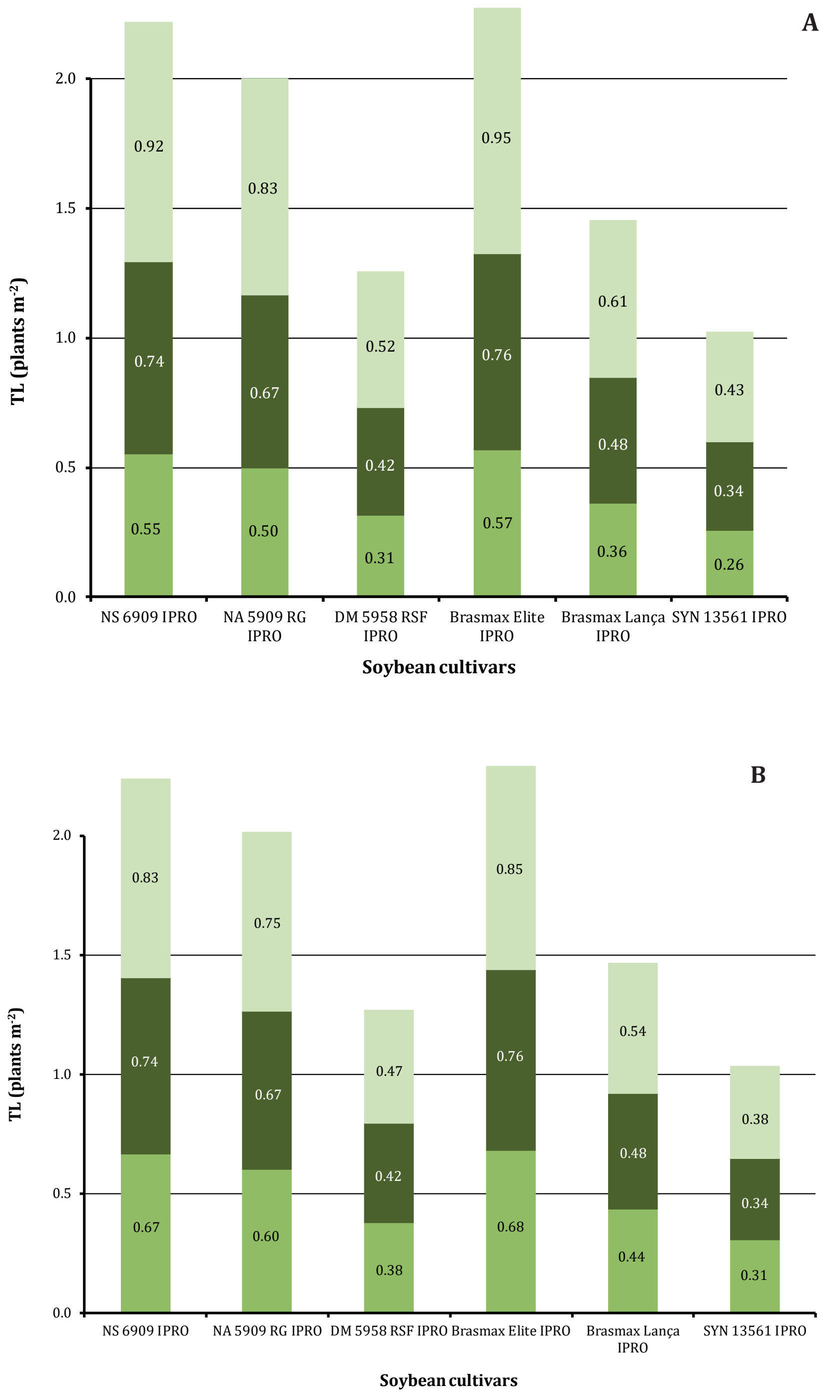


Figure 3. Threshold level (TL) of arrowleaf sida, *Sida rhombifolia*, as a function of control cost (A) and herbicide efficiency (B) and soybean cultivars. UFFS, Erechim-RS, 2017/18.

Figura 3. Nivel de daño económico (TL) de escoba dura (*Sida rhombifolia*) en función del costo de control (A) y de la eficiencia de los herbicidas (B) y de cultivares. de cultivares. UFFS, Erechim-RS, 2017/18.

The average results for all soybean cultivars with the highest versus the lowest price paid per bag exhibited 1.47 times higher variation in TL (figure 2B, page 102). Therefore, the lower the price paid per bag of soybeans, the higher the population of arrowleaf sida needed to overcome TL, thus compensating for the control method. Tavares *et al*. (2019) and Galon *et al.* (2019) reported similar results concerning the price paid per bag of wheat and soybean, respectively, corroborating the findings of this study.

The minimum cost for the average arrowleaf sida cost to control in all cultivars was 40.14% lower when compared with the maximum cost. Thus, the higher the cost of the control method, the higher the TL and the more arrowleaf sida plants per square meter needed to justify the control measures (figure 3A, page 103). The use of TL as a tool for weed management is only justified in farms that use good agricultural practices for soybean management, such as crop rotation, proper plant arrangement, use of more competitive cultivars, adequate sowing times, and correct soil fertilization.

For the efficiency of chemical control with herbicides, there were changes in the TL of 12.28 and 11.76% when comparing the average efficiency (90%) with the lowest (80%) and the highest (100%), respectively (figure 3B, page 103). Therefore, the control level influences TL, and the more efficient the herbicide, the lower the TL (the smaller the number of arrowleaf sida per square meter necessary to adopt the control measures). This was also verified by Agostinetto *et al*. (2010) and Galon *et al*. (2019), who applied herbicides to control barnyard grass and alexander grass in irrigated rice and soybean crops, respectively. Song *et al*. (2017) obtained TL of five common weed species assuming a 90% efficiency of the herbicide for the studied region of 0.70 plants m^{-2} as a threshold to control them in a soybean field, which corroborates with the results of this study.

Although the soybean cultivars differed from each other, the TL values indicated that the control was justified in low weed populations, even in situations where the soybean bag value was the lowest, exhibiting the high competitive capacity of this weed and the need to manage it to avoid losses. Konzen *et al*. (2021) reported that arrowleaf sida, when competing with soybean cultivars, reduced the dry mass and leaf area of crop plants, with interspecific competition being more harmful than intraspecific competition.

Considering the average soybean yield of 2,760 kg ha^{-1} for the last 10 years in the state of Rio Grande do Sul (9), an average price of $18.87 per 60 kg bag, and arrowleaf sida Cc of $17.07 ha^{-1}, we estimated that these costs were equivalent to 1.96% of production costs. The presence of 1 plant m^{-2} arrowleaf sida caused yield losses of 2.36%, 2.59%, 4.00%, 2.24%, 3.56%, and 4.85% in soybean cultivars NS 6909 IPRO, NA 5909 RG IPRO, DM 5958 RSF, Brasmax Elite IPRO, Brasmax Lança IPRO, and SYN 13561 IPRO, respectively, (table 1, page 99), and all cultivars exhibited losses higher than the control cost. These results indicate that arrowleaf sida are very competitive, even in low populations, and control measures that eliminate up to 99% of the infestation may not be sufficient to avoid losses in soybean grain yield.

When prices of agricultural products are high compared to the usual prices, the adoption of weed control measures with 100% effectiveness becomes important. Thus, even if only a few weeds remain in a given area, they will cause remarkable economic losses considering the product value, and small grain yield losses will result in a significant decrease in profits. Any stress can potentially be economically harmful.

Conclusions

The nonlinear regression model of the rectangular hyperbola adequately estimated the grain yield losses of NS 6909 IPRO, NA 5909 RG IPRO, DM 5958 RSF, Brasmax Elite IPRO, Brasmax Lança IPRO, and SYN 13561 IPRO soybeans in the presence of increasing arrowleaf sida densities and exhibited the best fit for the variable density of the arrowleaf sida plants. The soybean cultivars NS 6909 IPRO, NA 5909 RG IPRO, and Brasmax Lança IPRO were more competitive than DM 5958 RSF, Brasmax Elite IPRO, and SYN 13561 IPRO considering the explanatory variables (PD, LA, SC, and DM). The TL values varied from 0.55 to 0.95 plants m^{-2} for cultivars NS 6909 IPRO, NA 5909 RG IPRO, and Brasmax Lança IPRO, which proved to be more competitive with arrowleaf sida. The lowest TL values ranged 0.26-0.61 plants m^{-2} for the cultivars DM 5958 RSF, Brasmax Elite IPRO, and SYN 13561 IPRO, which had less competitive potential with weeds. The TL values decreased with an increase in grain yield and price of soybeans, a reduction in the cost of controlling arrowleaf sida, and herbicide efficiency, which justifies the adoption of control measures at lower weed densities.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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Advances in the etiology of sweet potato (*Ipomoea batatas* (L.) Lam) yellow curling disease in Argentina

Avances en la etiología de la enfermedad del encrespamiento amarillo de la batata (*Ipomoea batatas* (L.) Lam) en Argentina

Sofia Solange Flamarique *, Antonella Vilanova Perez *, Andrés Vicente Luque, Patricia Elsa Rodríguez Pardina, Liliana del Valle Di Feo

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Abstract

Sweet potato yellow curling (YC), the most severe disease of sweet potato detected in Argentina, causes symptoms and damage to sweet potato crops in all cultivated regions. Since 2010/11, the presence of four viruses has been detected in symptomatic cv. Arapey INIA: two potyviruses non-persistently transmitted by *Myzus persicae* (sweet potato feathery mottle virus, SPFMV and sweet potato virus G, SPVG)*;* a closterovirus, sweet potato chlorotic stunt virus (SPCSV) and a geminivirus, sweet potato leaf curl virus (SPLCV), both transmitted by *Bemisia tabaci* in a semi-persistent and persistent manner, respectively. All the plants were collected from fields in Colonia Caroya, Córdoba province, Argentina. The objectives of the present work are to isolate and identify the virus or viruses involved in YC disease of sweet potato, and to elucidate the viral combination that reproduces YC symptoms. The most severe YC symptoms for this genotype in the field were only reproduced by a combination of the four viruses. The symptoms include chlorosis, stunting, mosaic, blistering, leaf curling, chlorotic spots, chlorotic patterns, leaf area reduction and distortion, and upward curling of leaf edges. The presence of each virus was detected by serological (DAS, NCM and TAS-ELISA) and molecular (PCR) tests. It is concluded that the interaction of SPFMV, SPVG, SPCSV and SPLCV is needed for the development of YC symptoms.

Keywords

Ipomoea batatas • sweet potato feathery mottle virus • sweet potato virus G • sweet potato leaf curl virus • sweet potato chlorotic stunt virus • Arapey INIA • Koch's postulates

Unidad de Fitopatología y Modelización Agrícola (INTA-CONICET). Instituto de Patología Vegetal (IPAVE). Centro de Investigaciones Agropecuarias (CIAP). Instituto Nacional de Tecnología Agropecuaria (INTA).

* flamarique.sofia@inta.gob.ar / * vilanova.antonella@inta.gob.ar
* Both authors contributed equally to this study.

Resumen

El encrespamiento amarillo (EA), la enfermedad más severa detectada en Argentina, causa síntomas y daños en cultivos de batata en toda la región productora. Desde 2010/11 se ha detectado la presencia de cuatro virus en plantas sintomáticas del cv. Arapey INIA recolectadas en lotes de Colonia Caroya, provincia de Córdoba. Los virus son sweet potato feathery mottle virus (SPFMV) y sweet potato virus G (SPVG), dos potyvirus transmitidos de forma no persistente por *Myzus persicae*; un closterovirus: sweet potato chlorotic stunt virus (SPCSV) y un geminivirus: sweet potato leaf curl virus (SPLCV), ambos transmitidos por *Bemisia tacabi* de manera semipersistente y persistente, respectivamente. Los objetivos de este trabajo fueron aislar e identificar el o los virus involucrados en la enfermedad EA de la batata y determinar la combinación de virus que reproduce la sintomatología de EA Solo la combinación de los cuatro virus permitió reproducir la sintomatología más severa del encrespamiento amarillo observada a campo en dicho genotipo. Los síntomas incluyen clorosis, achaparramiento, mosaico, ampollado, enrulado de la hoja, manchas cloróticas, diseños cloróticos, reducción y distorsión del área foliar, bordes de la hoja curvados hacia arriba. La presencia de cada uno de los virus se detectó mediante pruebas serológicas (DAS, NCM y TAS-ELISA) y moleculares (PCR). Se concluye que la interacción de SPFMV, SPVG, SPCSV y SPLCV es necesaria para el desarrollo de EA.

Palabras claves

Ipomoea batatas • sweet feathery mottle virus • sweet potato virus G • sweet potato leaf curl virus • sweet potato chlorotic stunt virus • Arapey INIA • postulados de Koch

Introduction

Sweet potato, *Ipomoea batatas* (L.) Lam, is a perennial plant of the *Convolvulaceae* family, native to northwestern South America (6, 16). It is ranked among the 10 most important food crops worldwide, with a production of 89,487,835 tons in an area of 7,400,472 hectares in 2020 (8). The major sweet potato production areas are located in China and around the Great Lakes of East Africa, but the crop is also important in many other countries (6, 20). According to the International Potato Center, developing countries concentrate 95% of the production, but with low yields. Latin America produces only 1.5 million tons (8).

Viral diseases are considered a major constraint to sweet potato production worldwide (22, 38, 40), due to the vegetative propagation of this species and the consequent cumulative effect of virus infections (17, 29). The first report of a sweet potato viral disease in Argentina was published in the 1970s. This disease, named “batata crespa”, affected cv. Criolla Amarilla and was caused by the potyvirus sweet potato vein mosaic virus (SPVMV) (30). Later, sweet potato feathery mottle virus (SPFMV) was detected in the same cultivar (31). In 1978, Argentine farmers adopted a new cultivar, Morada INTA, tolerant to both potyviruses (SPVMV and SPFMV). However, during the 1980s, this new cultivar was affected by a severe disease called sweet potato chlorotic dwarf, caused by a synergistic combination of two aphid-transmitted potyviruses (SPFMV and sweet potato mild speckling virus, SPMSV) with a whitefly-transmitted closterovirus serologically related to sweet potato chlorotic stunt virus (SPCSV) (7). Sweet potato chlorotic dwarf was successfully controlled using propagation material obtained from disease-free areas. Nevertheless, in all sweet potato-producing areas of Argentina, cv. Morada INTA has been progressively replaced by cv. Arapey INIA, an Uruguayan genotype (7).

In 2010/11, a new viral disease with severe and variable symptoms was detected in this cultivar; it was named sweet potato yellow curling disease (YC). YC causes significant yield losses, with records of up to 90% loss in experimental trials and commercial plots. In addition, YC affects the quality of the storage roots (23).

Serological tests confirmed the presence of SPFMV (4) and SPCSV (Unpublished data) in symptomatic plants. Moreover, in these plants, two pathogens that had not been described in Argentina at that time, sweet potato virus G (SPVG), a potyvirus (33) and the geminivirus, sweet potato leaf curl virus (SPLCV) (34), were detected through serological tests and PCR, respectively.

Since YC is presumed to be caused by a viral complex, this work aimed to isolate and identify the virus or viruses involved in YC disease of sweet potato and to elucidate the viral combination that reproduces YC symptomatology.

Material and methods

Source of inoculum

More than 100 plants of sweet potato cv. Arapey INIA showing chlorosis, stunting, mosaic, chlorotic rings, chlorotic patterns, blistering, distortion, reduction of foliar area, and upward leaf curling were randomly collected from 12 fields in Colonia Caroya (31°01'16.8'' S 64°03'42.2'' W), Colón department, Córdoba province, Argentina. Plants were transplanted to pots and maintained in the greenhouse at 25°C under controlled conditions of humidity (70-75% RH) and photoperiod (16 h light) for further analysis.

Transmission assays

Apterous aphids from a healthy colony of *Myzus persicae* Sulz. were reared on *Raphanus sativus* L. and used for transmission studies. Fasted aphids were allowed to probe briefly (one probe) on plants of sweet potato cv. Arapey INIA showing typical YC symptoms. Aphids were transferred to healthy individuals of the indicator plant *Ipomoea setosa* (one aphid per each one of 44 plants) and allowed to feed for 12 h; then they were killed with an aphicide (Pirimicarb) (7, 13). A repetition was performed using sweet potato cv. Okinawa 100 plants infected with SPVG, SPCSV and SPLCV. The procedure was similar to the one described previously but involved 46 *I. setosa* plants.

To separate SPCSV from SPLCV (semi-persistent and persistent transmission, respectively), non-viruliferous *Bemisia tabaci* Gennadius whiteflies reared on poinsettia (*Euphorbia pulcherrima* Willd.), a sweet potato plant cv. Arapey infected with YC, and 42 healthy seedlings (second unfolded leaf) of *I. setosa* were placed in protected cages with anti-aphid mesh, at 25°C, in a greenhouse, under controlled conditions of humidity (70-75% RH) and photoperiod (16h light). The whiteflies were removed from the cages after 1h and up to 48h of exposure to insect feeding. At 21 days after inoculation, the plants were checked for the presence of SPCSV and SPLCV by TAS-ELISA and PCR, respectively (25).

Verification of SPFMV, SPVG, SPCSV and SPLCV presence

Infection by SPFMV, SPVG and SPCSV was checked by serological tests on all inoculated plants. A local antiserum was used for SPFMV detection. The SPVGas and SPCSVas were provided by S. Fuentes (International Potato Center, Lima, Peru) and H. J. Vetten (Federal Biological Research Center, Institute of Biochemistry, Agriculture, and Forestry, Braunschweig, Germany), respectively (7, 9). SPLCV was tested by PCR (34). The production of local SPVGas is discussed below.

SPVG virus purification and antiserum production

After SPVG isolation, virus particles were purified from *I. setosa*-infected leaves following the method described by Di Feo *et al.* (2000). The virus band was collected from a sucrose-CsCl step gradient (0 to 41% CsCl in borate buffer containing 20% sucrose) after ultracentrifugation (100000 x g for 5 h at 8°C), and dialyzed against 0.05 M borate buffer. Purified virus preparation of SPVG (0.02 mg/ml) was injected into a female New Zealand rabbit. Three inoculations were performed at 20-day intervals. The first inoculation (1ml of the purified virus + 2 ml of Freund's complete adjuvant) was administered by multiple intradermal injections, whereas the other two inoculations (1 ml of the purified virus + 1 ml of incomplete Freund's adjuvant) were administered intramuscularly. Blood sample collection started 20 days after the last injection. The titers were evaluated by Nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) (21, 32), Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (5) and Immunosorbent electron microscopy plus decoration (ISEM+D) (26).

To evaluate the optimal dilution to be used in NCM-ELISA, SPVG antiserum was serially diluted from 1:500 to 1:1024000 in Tris saline buffer + 2% skimmed milk. Samples were macerated in extraction buffer (TBS + 0.2% Na_2SO_3) in a 1/50 (w/v) proportion and blotted onto a nitrocellulose membrane. Positive controls were *I. setosa* plants infected with SPVG, sweet potato cv. Arapey INIA infected with YC and sweet potato cv. Okinawa infected with the complex (SPVG + SPCSV + SPLCV). Negative controls were healthy *I. setosa* and sweet potato plants. In addition, a sample of *I. setosa* infected with SPFMV was included to rule out the possible cross-reaction between the antiserum and this potyvirus.

For the DAS-ELISA test, IgG was purified from the SPVG antiserum. Enzyme conjugate was prepared with the purified IgG and Phosphatase Alkaline VII S (SIGMA -Aldrich) (10 mg protein/ml suspension of $SO_4(NH)_4$; 30000 enzyme units/ml). DAS-ELISA test was performed using a NUNC polystyrene plate with 96 flat-bottomed wells as support. Three dilutions of IgG (1:500, 1:1000 and 1:2000) were tested against three dilutions of enzyme conjugate (1:500. 1:1000 and 1:1500). Samples were macerated in extraction buffer (PBS + 0.05% Tween 20 + 2% PVP+ 2% skimmed milk + 0.2% SO_2Na_2) in a 1/10 and a 1/1000 proportion, respectively. Positive and negative controls were sweet potato plants infected with SPVG and healthy plants, respectively. Absorbance was measured using a spectrophotometer (Dynatech Laboratories, Model MRX) at 405 nm.

ISEM+D was performed according to the protocol described by Milne & Lesemann (1978). Copper grids were first covered with the SPVG-specific antiserum, diluted 1:1000 in TBS (20 mM Tris base, 500 mM NaCl, pH 7.5) and incubated for 30 min. Samples co-infected with SPVG + SPCSV were macerated in TBS containing 0.2% sodium sulfite (1/10 w/v). For decoration of virions, antiserum was diluted at 1:50 and incubated on grids for 15 min. Moreover, the modal length of virions obtained from purified preparations was determined. Observations were made under an electron microscope (JEOL JEM EX II 1220®).

Inoculation of healthy sweet potato plants with four viruses in different combinations

This experiment was designed to determine the viral combination(s) that reproduces YC in sweet potato. Scions of *I. setosa* plants infected individually with each of the four isolated viruses (SPFMV, SPVG, SPCSV and SPLCV) were double, triple and quadruple side-grafted onto 10 healthy plants of cv. Arapey INIA (obtained by "*in vitro*" meristem culture) and *I. setosa*, respectively. Six healthy plants of cv. Arapey INIA grafted with scions from YC-affected plants were used as controls.

Grafted plants were kept in protected cages with anti-aphid mesh in a greenhouse at 25°C under controlled conditions of humidity and photoperiod.

The presence of SPFMV was determined by NCM-ELISA using local antiserum (7). To detect SPCSV, a triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) was performed, as described by Gibson *et al.* (1998), using antisera provided by H. J. Vetten (Federal Biological Research Center, Institute of Biochemistry, Agriculture, and Forestry, Braunschweig, Germany). Infection with SPVG was confirmed by NCM-ELISA using the local antisera produced in this study. The presence of SPLCV was verified by PCR using the primers SPG1 and SPG2 designed to bind to conserved regions in open reading frames C2 and C1, and to amplify a 912-bp fragment (18).

Results

Transmission assay

Only SPFMV was transmitted to healthy *I. setosa* plants from sweet potato cv. Arapey INIA infected with the YC complex. However, the frequency of isolation using single aphids (*M. persicae*) was 77% for SPFMV and 13% for SPVG when the inoculum source was sweet potato cv. Okinawa 100 infected with SPVG, SPCSV and SPLCV. Virus sources were maintained by grafting onto *I. setosa* and sweet potato cv. Arapey INIA in insect-proof cages in the greenhouse.

Transmission of SPCSV and SPLCV from sweet potato plants infected with YC showed that one hour after the *I. setosa* seedlings were removed from the cage, 100% of the plants were infected with SPCSV and 0% with SPLCV. On the other hand, at 48 h, 100% of the plants were infected with SPLCV and 33% with SPCSV. Those plants negative for closterovirus were selected for further testing.

Virus purification and serology

SPVG was purified from single-infected *I. setosa* plants. The A_{260}/A_{280} ratio and viral concentration of the band extracted from the CsCl gradient were 1.16 and 0.161 mg/ml, respectively. According to the NCM-ELISA test, the optimal dilution of the antiserum was 1:512000, without cross-reaction with SPFMV.

In DAS-ELISA, optimal values of A_{405} were obtained after 90 minutes of reaction. The most suitable dilutions of IgG and enzyme conjugate for virus diagnosis were 1:1000 and 1:500, respectively (table 1), whereas 1/10 w/v was the most appropriate dilution of sweet potato plant tissue.

Table 1. Absorbance values (A405) in DAS-ELISA using SPVGas.

Tabla 1. Valores de absorbancia (A405) en DAS-ELISA utilizando SPVGas.

	IgG dilution (v/v)								
	1/500			1/1000			1/2000		
	IgG-AP conjugate dilution (v/v)								
	1/500	1/1000	1/1500	1/500	1/1000	1/1500	1/500	1/1000	1/1500
I. setosa infected with SPVG	1.038	0.549	0.363	0.967	0.504	0.285	0.667	0.374	0.205
Healthy plants	0.071	0.021	0.019	0.087	0.008	0.023	0.046	0.028	-0.012

When the SPVG antiserum was used for ISEM + Decoration tests, differentially decorated virions were observed in samples of tissue infected with SPVG +SPCSV (figure 1).

Observations were made under a transmission electron microscope (JEOL JEM EX II 1220®) (X150000).

Observaciones realizadas al Microscopio electrónico de transmisión (JEOL JEM EX II 1220®) (X150000).

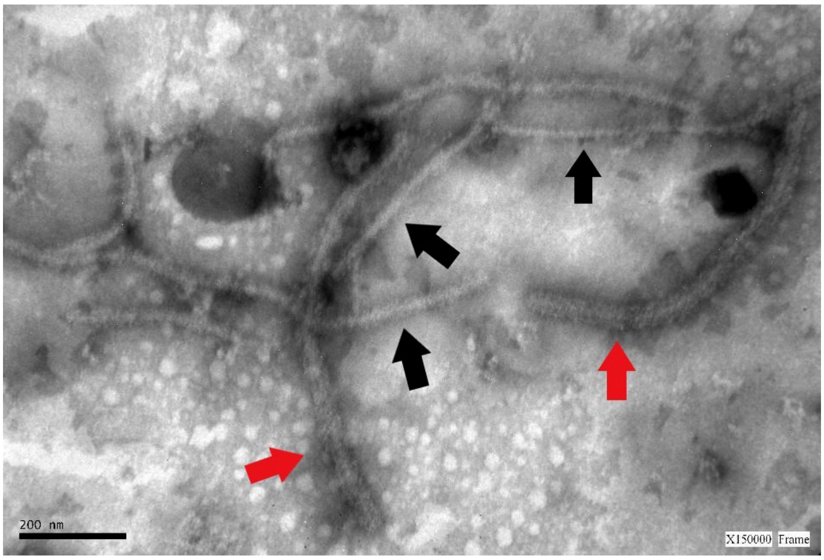


Figure 1. Virus particles from tissue samples co-infected with SPVG + SPCSV. ISEM+D coated 1/2000 with SPVG antiserum, decoration 1/50 (v/v), and contrasted with 2% uranyl acetate (red arrow).

Figura 1. Viriones de SPVG de muestras de tejido coinfectado con SPCSV, ISEM + D sensibilizado con antisuero SPVG diluido 1/2000, decorado con antisuero diluido 1/50 (v/v), y contrastado negativamente con acetato de uranilo al 2% (flecha roja).

A modal length of 850-900 nm (150 virions) from purified suspensions was determined (figure 2, page 112), which corresponds to the range of length established for potyviruses (37).

The modal length (850-900 nm) corresponds to the range of length established for potyviruses.

La longitud modal (850-900 nm) corresponde al rango de longitud establecido para los potyvirus.

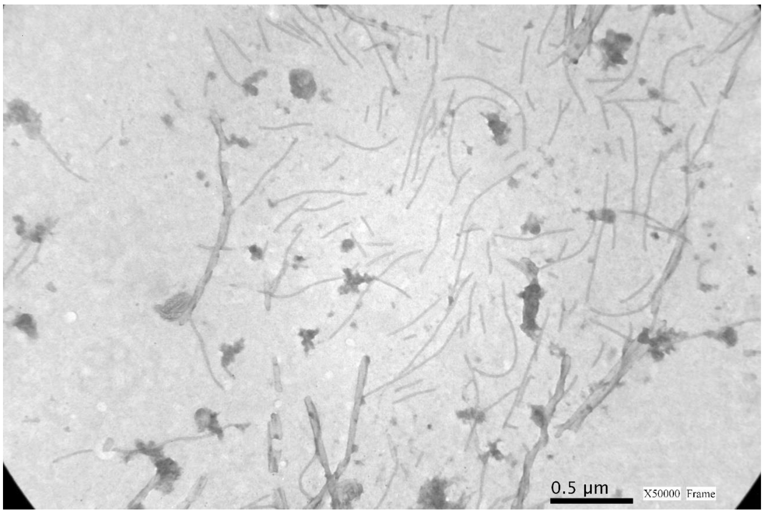


Figure 2. SPVG viral particles from purified suspensions, observed under a transmission electron microscope JEOL JEM EX II 1220® (X50000).

Figura 2. Partículas virales SPVG observadas al microscopio electrónico de transmisión JEOL JEM EX II 1220® a partir de suspensiones purificadas (X50000).

Inoculation

Symptoms caused by each virus combination are shown in table 2. The mixed infections in which SPCSV was present caused different degrees of severity.

Table 2. Symptoms induced by different combinations of sweet potato feathery mottle virus (SPFMV), sweet potato virus G (SPVG), sweet potato chlorotic stunt virus (SPCSV) and sweet potato leaf curl virus (SPLCV) in graft-inoculated *Ipomoea setosa* and sweet potato (*Ipomoea batatas*) cv. Arapey INIA.

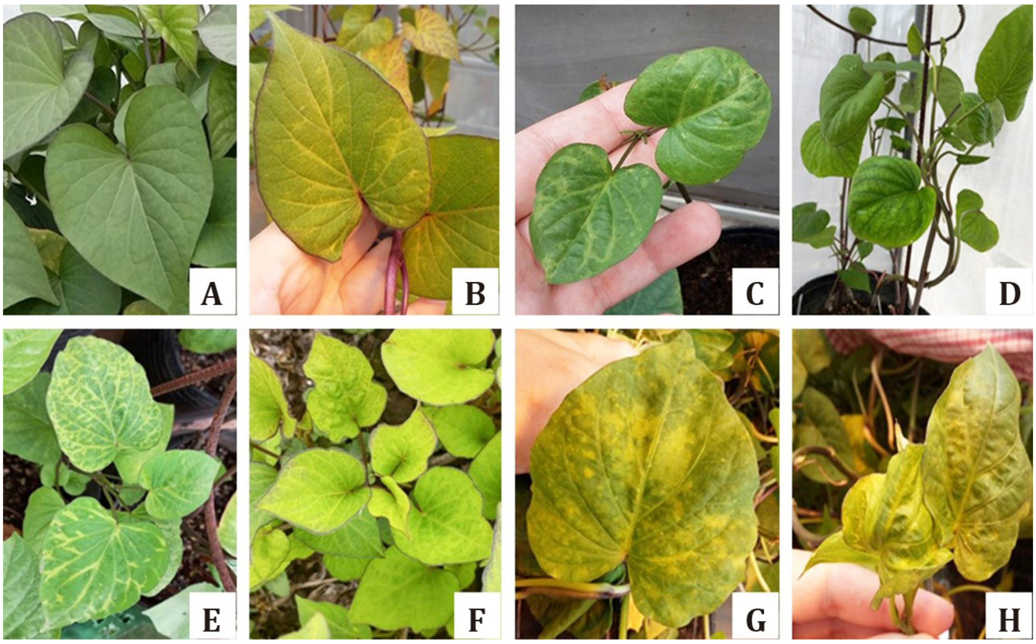
Tabla 2. Síntomas inducidos por diferentes combinaciones de sweet potato feathery mottle virus (SPFMV), sweet potato virus G (SPVG), sweet potato chlorotic stunt virus (SPCSV) y sweet potato leaf curl virus (SPLCV) en plantas de *Ipomoea setosa* y batata (*Ipomoea batatas*) cv. Arapey INIA inoculadas mediante injerto.

Host	Virus	Symptoms
I. setosa	SPVG +SPFMV	Mosaic, feathery mottling, vein clearing, chlorotic spots and leaf distortion
	SPFMV + SPCSV	Mosaic, feathery mottling, chlorotic spots, blistering and severe leaf distortion (“shoelace”)
	SPFMV + SPLCV	Feathery mottling in lower leaves and upward curling of margins of upper leaves
	SPVG + SPLCV	Mosaic and vein clearing in upper and lower leaves and upward curling of margins of upper leaves
	SPVG + SPCSV	Severe mosaic, vein clearing, and severe leaf distortion (“shoelace”) in upper leaves.
	SPCSV + SPLCV	Mosaic, blistering, leaf distortion with upward curling of margins in upper and lower leaves
	SPVG + SPFMV + SPCSV	Mosaic, feathery mottling, vein clearing and severe leaf distortion (“shoelace”) in upper and lower leaves
	SPVG + SPFMV + SPLCV	Feathery mottling and mosaic in lower and upper leaves and curling of margins in upper leaves
	SPVG + SPCSV + SPLCV	Mosaic, vein clearing and vein banding and shoelace in upper leaves
	SPFMV + SPCSV + SPLCV	Mosaic, vein clearing and feathery mottling and shoelace in upper leaves
	SPFMV + SPVG + SPCSV + SPLCV	Mosaic, vein clearing, feathery mottling, blistering and severe leaf distortion (“shoelace”)

Host	Virus	Symptoms
I. batatas	SPVG +SPFMV	Mild mosaic, chlorotic spots, vein clearing in upper leaves
	SPFMV + SPCSV	Severe leaf distortion ("shoelace"), mosaic and vein clearing
	SPFMV + SPLCV	Mosaic and feathery mottling, and upward curling of margins of upper leaves
	SPVG + SPLCV	Mild mosaic and vein clearing, upward curling of margins of upper leaves
	SPVG + SPCSV	Vein clearing, chlorosis, blistering and vein banding. Severe leaf distortion ("shoelace") in upper leaves
	SPCSV + SPLCV	Mild mosaic and upward leaf margins
	SPVG + SPFMV + SPCSV	Chlorotic spots in upper and lower leaves. Vein clearing and feathery mottling, mosaic
	SPVG + SPFMV + SPLCV	Mosaic, vein clearing and thickening, blistering, upward leaf margins
	SPVG + SPCSV + SPLCV	Vein clearing, chlorosis, mosaic, blistering and vein banding. Severe leaf distortion ("shoelace") in upper leaves
	SPFMV + SPCSV + SPLCV	Severe leaf distortion ("shoelace"), mosaic and vein clearing, upward curling of margins of upper leaves
	SPFMV + SPVG + SPCSV + SPLCV	Chlorosis, stunting, mosaic, blistering, leaf curling, chlorotic spotting, chlorotic patterns, reduction and distortion of leaf area ("shoelace"), upward curling of leaf margins

The combination of the four viruses induced the most severe symptomatology, resembling that of YC-affected plants in the field (figure 3): chlorosis, stunting, mosaic, blistering, leaf curling, chlorotic spotting, chlorotic patterns, reduction and distortion of leaf area, and upward curling of leaf margins. Young plants of cv. Arapey INIA developed this symptomatology 15 days after grafting with the combination of the four viruses.

On the other hand, *I. setosa* became symptomatic 10 days after inoculation. Symptoms varied from mosaic, vein clearing, feathery mottling and blistering to severe leaf distortion ("shoelace").



(A) Asymptomatic leaf (healthy plant); (B) SPFMV + SPVG, feathery mottle and chlorotic spots; (C) SPFMV + SPVG + SPCSV, interveinal chlorosis, chlorotic spots and upward curling of leaf margins; (E-H) SPFMV + SPVG + SPCSV + SPLCV, feathery mottle, mosaic, vein clearing, chlorotic spots, blistering, upward curling of leaf margins, leaf area reduction and distortion.

(A) Hoja asintomática (planta sana); (B) SPFMV + SPVG, moteado plumoso y punteado clorótico; (C) SPFMV + SPVG + SPCSV, clorosis internerval, punteado clorótico y curvado de hojas; (E-H) SPFMV + SPVG + SPCSV + SPLCV, moteado plumoso, mosaico, aclaramiento de nervaduras, punteado clorótico, ampollado, curvado de hojas, reducción y distorsión del área foliar.

Figure 3. Symptoms induced in cv. Arapey INIA by different combinations of the viruses involved in sweet potato yellow curling disease.

Figura 3. Síntomas observados en diferentes combinaciones de los virus involucrados en encrespamiento amarillo cv. Arapey INIA.

Discussion

Vegetative propagation is a cultural practice that facilitates efficient virus perpetuation and dissemination way between cropping seasons or growing areas (14). While several viral diseases have been reported in sweet potato, YC is the most severe one reported in Argentina so far. It is caused by at least four viruses and produces severe symptoms and significant damage in all growing areas in the country (23).

The new disease differs from the ones previously described in Argentina. Both "batata crespa" and "sweet potato chlorotic dwarf disease" affected only sweet potato plots from Santiago del Estero and Córdoba provinces. The former was caused by SPVMV, and the latter, by three viruses: SPCSV + SPFMV + SPMSV (7, 30). Two viruses already present in Argentina (SPFMV and SPCSV) and two new viruses (SPVG and SPLCV) are involved in YC.

Mixed viral infections and synergistic complexes are frequent in sweet potato (36). SPFMV is the most common virus infecting sweet potato and occurs in all sweet potato growing regions (27). SPCSV interacts synergistically with SPFMV to cause sweet potato virus disease (SPVD), the most serious disease of sweet potato (1, 12, 19, 29). The ubiquitous presence of SPFMV has often masked the presence of other viruses in sweet potato, especially those belonging to the same family, such as SPVG; thus, detecting or isolating them is very difficult (35). It is widely known that suitable management of viral diseases that affect the sweet potato crop requires rapid and accurate detection (6). However, in previous works, it has been shown that low titers of the viral agents and the high concentration of inhibitors in the sweet potato plant hinder serological and molecular diagnosis (11, 14). The antiserum obtained allowed us to detect SPVG in single and mixed infections. In the latter case, the detection was evident, without cross-reaction with SPFMV, a ubiquitous virus in sweet potato crops (39). Detection reagents are available for local species involved in YC, such as SPVGas, which is used for routine diagnosis in IPAVE.

On the other hand, geminiviruses that infect sweet potato are widely distributed throughout the world. Twelve viruses belonging to the *Geminiviridae* family have been reported (6, 15, 28). They can cause significant losses in the production and quality of certain sweet potato genotypes, without noticeable symptom expression (41). SPLCV has been detected in several locations worldwide, including Taiwan, Japan, Israel, and the United States. The geographical range of this virus, however, is still mostly unknown (14). Leaf curl disease associated with SPLCV was first reported in Argentina in 2012 (34). Sweet potato leaf curl Georgia virus (SPLCGV) was also reported in Argentine sweet potatoes (24). The presence of both pathogens is associated with an increase in whitefly populations (3). Therefore, it is necessary to study the dispersion of the new viral species, since climate change causes the geographical expansion of vectors such as whiteflies (2, 3, 10).

The global exchange of sweet potato germplasm contributes to the wide distribution of the viruses in the sweet potato production regions (19). One effective way to prevent the spread of viruses and, therefore, control viral diseases is to use virus-tested planting material.

The new disease, called yellow curling, is a serious threat to sweet potato crops in Argentina. We are conducting studies to demonstrate its detrimental effects on the production and quality of roots.

Conclusion

In this work, the first antiserum for the fast, safe and efficient diagnosis of SPVG in the country was obtained.

The four viruses involved in YC disease were isolated and identified: SPFMV, SPVG, SPCSV and SPLCV.

The interaction of SPFMV, SPVG, SPCSV and SPLCV is needed for the development of yellow curling symptoms in sweet potato in Argentina.

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Responses of *Panicum* and *Brachiaria* to irrigation during winter in the Goiás' Cerrado-Brazil

Respuestas de *Panicum y Brachiaria* al riego durante el invierno en el Cerrado de Goiás-Brazil

Dyb Youssef Bittar [1], Wilian Henrique Diniz Buso [2], Cleiton Mateus Sousa [2*]

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Abstract

Forage production in the Cerrado is based on well-defined seasons: a rainy summer, and a dry winter. To define strategies that minimize the seasonality of forage production, responses in growth and biomass accumulation of five irrigated forages were evaluated during winter. The experimental design was randomized blocks, in a split plot arrangement, with five replicates, five forages in the plots (three of *Panicum maximum* - Mombasa, Tanzania, and Masai; two of *Brachiaria brizantha* - piatã and MG5), and five cuts in the subplots made from July to October 2016. Forages were cultivated with centre-pivot irrigation and the water level was defined according to the reference evapotranspiration determined through a Class A pan evaporation method, 0.8 kc, and two-day irrigation shifts. The number of shoots, leaves/tiller, leaves/plant, fresh leaf and stem biomass, dry leaf and stem biomass, fresh leaf mass/fresh stem mass ratio, dry leaf mass/dry stem mass ratio, and biomass accumulated in the five cuts were calculated. Irrigated forage during winter in the Goiás' Cerrado showed satisfactory growth, reaching a lower plant height between cuts than those reported in the literature. Among the *Panicum* grasses, Mombasa grass stood out, and MG was superior to piatã grass.

Keywords
forage • fresh weight • dry weight • tillering • supplementary irrigation

1 Mestre em Irrigação no Cerrado. Instituto Federal Goiano. Campus Ceres. Rod. GO 154, km 03. Zona Rural. Ceres-GO. 76.300-000. Brazil.
2 Instituto Federal Goiano. Campus Ceres. Rod. GO 154, km 03, Zona Rural. Ceres-GO. 76.300-000. Brazil. * cleiton.sousa@ifgoiano.edu.br

Resumen

La producción de forrajes en el Cerrado presenta estacionalidad debido a estaciones bien definidas, una lluviosa, con altas temperaturas y días largos, y la otra, estación seca, con temperaturas más bajas y días más cortos. Con el fin de definir estrategias para minimizar la estacionalidad en la producción de forrajes, se evaluaron las respuestas de crecimiento y acumulación de biomasa en cinco forrajes regados durante el invierno en Cerrado Goiano. El diseño experimental utilizado fue en bloques al azar, en un arreglo de parcelas divididas, con cinco repeticiones, cinco forrajes en las parcelas (tres del género *Panicum maximum* - mombaça, tanzânia y massai; dos del género *Brachiaria brizantha* - piatã y MG5) y cinco cortes en las subparcelas. Los cortes se realizaron entre julio y octubre de 2016. Los forrajes se cultivaron en un área irrigada con pivote y la lámina de agua se definió según la evapotranspiración de referencia (ETo), estimada por el tanque clase A, 0.8 kc y riego con un intervalo de dos días. Se evaluó el número de macollos, hojas por labranza, hojas por planta, biomasa de hojas y tallos frescos, biomasa seca de hojas y tallos, relación masa de hojas frescas x masa fresca, relación masa de hojas secas x masa seca de los cinco cortes. Los forrajes regados durante el invierno en el Cerrado Goiano mostraron un crecimiento satisfactorio, alcanzando altura de planta con intervalos de corte más cortos que los encontrados en la literatura. Mombaça se destacó entre el Panicum y MG fue superior al piatã.

Palabras clave

forraje • materia fresca • materia seca • macollamiento • riego complementario

Introduction

The Goiás' Cerrado has a seasonal tropical climate with rainy summers and dry winters. The temperature can reach 40°C in summer and 10°C in winter. The average annual rainfall is approximately 1500 mm, and is distributed between October and April. In winter, the days are shorter, whereas in summer, they are longer.

It is estimated that the Cerrado has 50 million ha of cultivated pastures (24). In the pastures in the Goiás' Cerrado, grasses of the genus *Brachiaria* are predominantly grown (5, 18); however, the areas cultivated with *Panicum* have been significantly increasing. These plants have a C4 carbon fixation metabolism and generally exhibit better growth and biomass accumulation at temperatures between 30 and 40°C and high luminosity.

The areas of pastures cultivated with *Brachiaria* species in Brazil are probably unequalled by other forages in any other country with a tropical climate, especially at the national level with *Brachiaria brizantha* cv. MG-5 (7). On the other hand, *Panicum maximum* is one of the main forage species cultivated worldwide, and is highly valued for its dry matter production and forage quality (23).

In tropical regions, rain is the main factor that limits the growth and accumulation of forage mass, and it has been verified that the seasonality of rainfall influences the increase in biomass of *Pennisetum purpureum* cv. Roxo (16). The seasonality of *Brachiaria brizantha* cv. Marandu production, even with fertigation, had higher values in spring and summer and lower values during the autumn-winter season (22).

Nevertheless, the seasonality in the growth and accumulation of biomass depends on the interaction of the plants with edaphoclimatic conditions, and in some cases, the water supply does not provide the maximum forage yield. In these cases, the low yields in autumn-winter were compensated for by the better quality of forage crude protein and neutral detergent fiber (22).

The use of irrigation in pastures in the Cerrado still predominates based on the empirical experience of producers and is still not based on academic research. The authors found that dry matter production of Mombasa grass during spring was higher than that during winter, with an increase in the production of dry matter with an increase in the minimum temperature. They also defined minimum air temperature and water availability in the soil as the main factors responsible for the mass production of Mombasa grass.

Higher air temperatures, with high luminosity and moisture, stimulate the growth of C4 plants throughout the year, providing high productive potential. However, low temperatures can reduce or even prevent plant growth. Little or no growth is expected for tropical grasses when temperatures are below 15°C (17). Thus, even with irrigation, the plant did not produce good yields under these conditions (20).

Accordingly, the objective of this study was to evaluate the responses of growth and biomass accumulation of five forage plants grown under irrigation in the Goiás' Cerrado.

Material and methods

The experiment was conducted at the Federal Institute Goiano - Campus Ceres, Ceres - GO, in a center-pivot irrigated plot, with the following Cartesian Coordinates: 15°18′49" S, 49°36´12" W, located at an approximate altitude of 570 m a. s. l.

According to Koeppen's classification, the climate of the site is Aw, which is a tropical climate with a dry season in winter. Average minimum temperature in the coldest months was below 15°C, and in the hottest months, the average maximum temperature exceeded 35°C. The average annual rainfall is approximately 1575 mm.

The soil of the experimental area is a typical eutrophic Red Latosol with a very frank clayey texture. The chemical and physical properties of the soil in the experimental area are: 48.2% sand, 4.0% silt, 47.8% clay, pH (in water) = 5.62, organic matter (OM) = 22 g dm^{-3} (colorimetric), P = 50.0 mg dm^{-3}, K = 0.56 cmolc dm^{-3}, Ca = 3.85 cmolc dm^{-3}, Mg = 1.94 cmolc dm^{-3} (KCl $mol.l^{-1}$), $H^{+}Al$ = 3.80 cmolc dm^{-3} (SMP buffer at pH 7.5), and V = 62.57%. The methodology used for the soil analysis followed the recommendations of Embrapa (2011).

The average, maximum, and minimum temperatures, and evapotranspiration (figure 1) were determined using the Class A pan evaporation method during the experimental period.

During the experiment, the following rainfall was recorded: 16/08/2016 - 42 mm, 21/08/2016 - 1 mm, 25/08/2016 - 1 mm, 01/09/2016 - 20 mm, 02/09/2016 - 2 mm, 16/09/2016 - 10 mm, 17/09/2016 - 1 mm, 25/09/2016 - 25 mm, 01/10/2016 - 18 mm, 03/10/2016 - 1 mm, 04/10/2016 - 15 mm, 05/10/2016 - 12 mm, and 06/10/2016 - 6 mm.

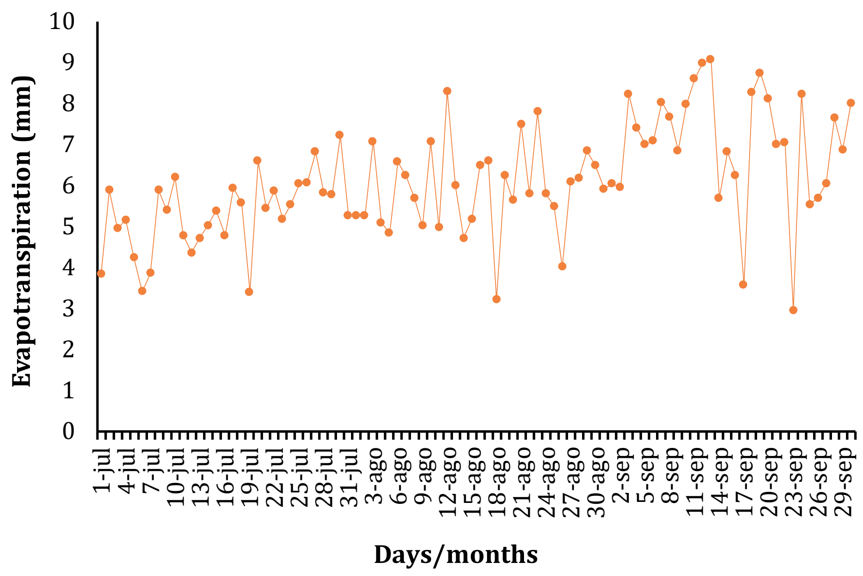

Figure 1. Evapotranspiration monitored by Class A pan evaporation method during the experimental period.

Figura 1. Evapotranspiración monitoreada por el método de evaporación, tanque Clase A, durante el período experimental.

The experimental design was a completely random block split plots arrangement with five replicates, five forages in the plots (three of the species *Panicum maximum* - Mombasa, Tanzania, and Masai, two of the species *Brachiaria brizantha* (cultivar piatã and MG5), and five cuts in the sub-plots.

The soil was prepared in a conventional manner with one plowing and two harrowings. The deployment of forage occurred on December 29, 2015, with a distribution of 20 kg of seeds per ha, with a crop value of 50% for *Brachiaria* and 32% for *Panicum*.

Seventy days after emergence, the plants were cropped to standardize the plots, and the residues were removed, followed by application of the treatments.

The area was irrigated with a central pivot, and the water level was calculated according to the reference evapotranspiration (ET0) from a class A pan, crop coefficient of 0.80 (1), and two-day irrigation shifts.

Maintenance fertilization was performed with urea, which provided 300 kg N ha^{-1} $year^{-1}$ for Mombasa and Tanzania, 250 kg N ha^{-1} $year^{-1}$ for Masai, and 200 kg N ha^{-1} $year^{-1}$ for MG5 and Piatã. The distribution of fertilizer was always by haul after cropping.

The sward height was monitored twice per week. When the grasses displayed the height indicated in the literature (Mombasa 0.9 m; Tanzania 0.7 m; Masai 0.55 m; MG 5 and piatã 0.35m), the forage plants were cropped, retaining the height recommended in the literature.

Sampling was conducted on July 30, August 18, September 05, September 21, and October 07 for Mombasa, Tanzania, and Massai grass, and August 02, August 20, September 07, September 23, and October 9 for MG5 and Piatã grass, all in 2016.

For each cut, the number of tillers, leaves per tiller, leaves per plant, fresh biomass of the leaf and stem, dry leaf biomass and stem, fresh mass ratio of leaves relative to that of fresh mass, and ratio of the dry mass of leaves relative to that of dry mass of stems were evaluated. The biomass accumulated in the five cuts was estimated using the fresh and dry biomass obtained from each cut.

The results were subjected to analysis of variance (F-test at 5%), and the means were compared using the Tukey test with 5% significance using the statistical program SISVAR.

Results

During the experimental period, the maximum, minimum, and average temperatures, photoperiod, total light exposure, volume of water applied, and photothermal units, and consequently, the intervals between the cuts, were varied (table 1). The shortest interval between cuts was 15 days, whereas the longest was 22 days, a difference of 46.6%. This demonstrates that the environmental conditions in the Goiás' Cerrado, besides water, influence forage growth, which is in agreement with the findings from existing literature.

Table 1. Intervals between cuts, maximum, minimum, average temperatures, photoperiod, total light exposure, volume of water applied, and photothermal unit during the experiment. Ceres-GO. 2016.

Tabla 1. Intervalos entre cortes, máxima, mínima, temperaturas promedio, fotoperiodo, exposición total a la luz, volumen de agua aplicado y unidad fototérmica durante el experimento. Ceres-GO. 2016.

Cuts	Days/months		Interval (days)	Air temperature (°C)			Photoperiod		Total light (h)	Volume of water (mm)	Photo-thermal unit
	Beginning	End		Maximum	Minimum	Average	Beginning	End			
1	08/07	07/30	22	30.33	17.08	23.71	11h12min	11h23min	248.1	87.25	185.0
2	07/30	18/08	20	33.09	19.01	26.01	11h23min	11h36min	229.6	84.98	208.0
3	18/08	05/09	21	32.97	18.39	25.68	11h36min	11h51min	211.1	87.73	209.5
4	05/09	09/20	15	35.06	20.66	27.86	11h51min	12h06min	179.5	94.06	193.0
5	09/20	07/10	17	35.58	22.03	28.85	12h06min	12h19min	207.6	77.77	205.0

Regarding the number of tillers, in each cut, variation was observed among forages, with Masai standing out among the *Panicum* and no significant differences were found between the *Brachiaria*. Among the cuts, a significant difference ($p < 0.05$) was observed only for piatã grass (table 2).

Table 2. Number of tillers in forage plants of *Panicum* and *Brachiaria* during five cuts.

Tabla 2. Número de macollos en plantas forrajeras del género *Panicum* y *Brachiaria* durante cinco cortes.

Forage	Cuts**				
	1	2	3	4	5
Panicum					
Masai	11.54 Aa*	12.70 Aa	11.15 Aa	13.39 Aa	11.24 ABa
Mombasa	7.11 Ba	8.57 Ba	7.00 Ba	6.30 Ca	7.04 Ca
Tanzania	6.91 Ba	6.45 Ba	8.35 ABa	7.82 BCa	7.73 CBa
Brachiaria					
MG5	11.06 Aa	13.14 Aa	10.80 ABa	11.04 ABa	12.59 Aa
Piatã	11.24 Aa	7.13 Bb	10.64 ABab	11.89 Aa	12.27 Aa

* Means in the rows followed by the same letter and means in the column followed by the same letter do not differ by Tukey's test at 5% significance.

* Medias en las filas seguidas por la misma letra y medias en la columna seguida por la misma letra no difieren por la prueba de Tukey al 5% de significación.

** Dates of cuts of Mombasa, Masai, and Tanzania grasses: 30/07/2016; 18/08/2016; 05/09/2016; 20/09/2016; and 07/10/2016. Dates of cuts for MG5 and Piatã 02/08/2016; 20/08/2016; 07/09/2016; 23/09/2016; and 09/10/2016.

** Día de corte de Mombasa, Masai y Tanzania: 30/07/2016; 18/08/2016; 09/05/2016; 20/09/2016; y 10/07/2016. Fechas de cortes para MG5 y Piatã 08/02/2016; 20/08/2016; 09/07/2016; 23/09/2016; y 10/09/2016.

The number of leaves per tiller of forage grass was not significantly different. Regarding the number of leaves per plant, Masai was superior to Mombasa and Tanzania, whereas no differences were observed between the *Brachiaria* grasses (table 3). In relation to the total fresh and dry weights of aerial parts, Mombasa grass stood out among the *Panicum* grasses, whereas MG5 was superior to piatã among the *Brachiaria* grasses (table 3).

Table 3. Number of leaves per tiller (NLPT), number of leaves per clump (NLPC), fresh biomass (FBM), and dry biomass (DBM) between cuts in forage plants of the genus *Panicum* and *Brachiaria.*

Tabla 3. Número de hojas por macollo (NLPT), número de hojas por racimo (NLPC), biomasa fresca (FBM) y biomasa seca (DBM) entre cortes en plantas forrajeras del género *Panicum* y *Brachiaria*.

Forage	NLPT	NLPC	FBM (t ha^{-1})	DBM (t ha^{-1})
Panicum				
Masai	2.43 A*	28.08 A	7.73 C	2.01 B
Mombasa	2.50 A	17.50 B	11.34 A	2.52 A
Tanzania	2.55 A	18.49 B	8.40 B	1.77 B
Brachiaria				
MG5	1.57 A	18.57 A	9.61 A	3.02 A
Piatã	1.76 A	19.08 A	6.67 B	2.12 B

* Means in the column followed by the same letter do not differ according to Tukey's test at 5% significance.

* Medias en la columna seguida de la misma letra no difieren por la prueba de Tukey al 5% de significación.

The ratio of fresh leaf mass to fresh stem mass did not vary among the forage grasses. In turn, regarding the ratio of dry leaf mass to dry stem mass, Masai grass was superior to Mombasa and Tanzania grass, whereas MG5 and piatã grass did not differ. The fresh and dry masses varied between cuts. The highest fresh mass was obtained in the cutting with higher average, maximum, and minimum temperatures and photoperiods. Dry mass did not show this trend (figure 2).

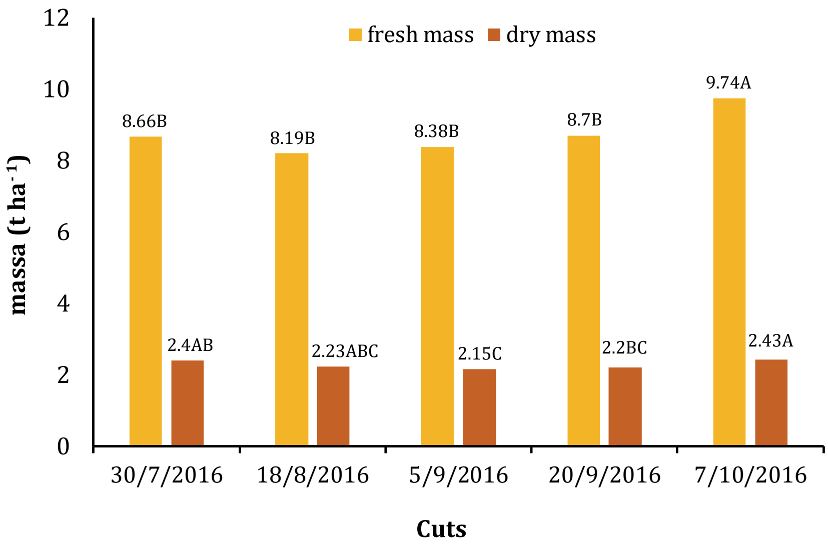

Figure 2. Accumulation of fresh and dry biomass (t ha^{-1}) among the forages in each cut.

Figura 2. Acumulación de biomasa fresca y seca (t ha^{-1}) entre los forrajes de cada corte.

Mombasa grass displayed a higher accumulated fresh mass in the five cuts, whereas MG5 grass had a higher accumulated dry mass in the five cuts (table 4). The ratio of dry mass to fresh mass of forage ranged from 20 to 32.41%. The highest percentage was obtained with MG5 grass, and the lowest with Tanzania grass. For the grasses of the genus *Panicum*, this ratio ranged between 20 and 26%, and for *Brachiaria*, the ratio was above 32% (table 4). The ratio of leaf tissue to total biomass was more than 90% in forage plants of the genus *Panicum* and between 71% and 73% in *Brachiaria* (table 4).

Table 4. Total fresh biomass (TFBM) and total dry biomass (TDBM) accumulated in the five cuts, percentage of dry biomass (% DBM), percentage of leaves (%), and percentage of stems (%) in forage grown in the winter under irrigation in the Goiás' Cerrado.

Tabla 4. Biomasa fresca total (TFBM) y biomasa seca total (TDBM) acumulada en los cinco cortes, porcentaje de biomasa seca (% DBM), porcentaje de hojas (%) y porcentaje de tallos (%) en forraje cultivado en el invierno bajo riego en el Cerrado de Goiás.

Forage	TFBM (t ha^{-1})	TDBM (t ha^{-1})	DBM (%)	Leaves (%)	Stem (%)
Masai	38.72 D	10.11 CD	26.06	97	3
MG5	46.67 B	15.13 A	32.41	73	27
Mombasa	62.29 A	12.59 B	20.21	91	9
Piatã	32.54 E	10.62 C	32.63	71	9
Tanzania	41.93 C	8.45 D	20.10	94	6

* Means in the rows followed by the same letter and means in the column followed by the same letter do not differ by Tukey's test at 5% significance.

* Medias en las filas seguidas por la misma letra y medias en la columna seguida por la misma letra no difieren por la prueba de Tukey al 5% de significación.

Discussion

Forage irrigation in winter provided satisfactory growth, overcoming the limitations of growth and biomass production and allowing forage production to be scaled up in the Central-West region of Brazil (15).

The forages studied were C4 plants, which have a higher production of photoassimilates and, consequently, higher growth, at air temperatures between 30 and 40°C and with higher luminosity. When temperatures are between 10 and 15°C, the growth of tropical grasses is hindered; therefore a standard base temperature of 15°C was adopted for the growth of these plants. However, there are differences between species and cultivars. Moreno *et al.* (2014) estimated the base temperatures to be 16°C for Masai, 11°C for Mombasa, and 7°C for Tanzania grass. For *Brachiaria* MG5 and Piatã, there is no published information on base temperatures, whereas for other *Brachiaria*, it is reported as above 16°C.

Brachiaria MG5 grew to a height above 35 cm, with intervals between cuts maintained to be at least 20 days (7). For plants of the genus *Panicum*, the interval was approximately 28 d. Temperature was not a limiting factor for the growth of the forages studied, and the irrigation of forage during the winter in the Goiás' Cerrado yielded plant heights at intervals between the croppings that were below published values (4).

In the Southeast and Central-West regions of Brazil, the predicted herbage accumulation rate of *P. maximum* increased from October onwards, reached its highest values in January (100-120 kg DM ha^{-1} d^{-1}), and decreased again in May (19). However, with irrigation, it was possible to anticipate the growth of fodder during winter in Ceres, Goiás, Brazil.

Prado *et al.* (2014) did not observe any changes in the population density of tillers as a function of the intensity and frequency of defoliation, whereas the number of tillers increased with increasing P in a greenhouse (10).

In studies conducted with Masai grass, the number of leaves per tiller was higher, ranging from 4.1 to 5.4 with 0 and 160 kg N ha^{-1} $year^{-1}$, respectively (8). The number of green leaves in Mombasa grass responded to N doses, reaching values of 5.90 leaf $tiller^{-1}$ with 269 kg N ha^{-1} (3). Decreases in temperature and photoperiod decreased the fresh and dry mass, height of aerial parts, density of tillering, leaf area, chlorophyll content, and relative water content in *Cynodon dactylon* (12).

The photoperiod and temperature influenced the productivity of Tanzania grass, even without a water deficit (21). The production of fresh and dry mass in Mombasa and Masai grass was lower in months with lower temperatures and shorter days (13). Piatã and MG5 grasses also presented lower yields of green and dry biomass in the months with shorter days and lower temperatures (19). This pattern was not observed in the accumulation of dry mass under the conditions of the Goiás' Cerrado with the use of pivot irrigation, indicating a way to minimize seasonality in the production of these forages under these conditions.

In the evaluation of the six forage grasses managed by grazing under the effect of different nitrogen doses and annual seasons, MG5, Mombasa, and Tanzania grass had higher rates of fresh biomass and dry matter (2). This divergence in the responses of forage indicates the need to evaluate and select plants that are more promising for specific climatic conditions in each region (6).

A ratio of 19 to 23% was observed between the culture seasons with *Panicum* grass (23). The ratio of dry mass to fresh mass in *Brachiaria ruziziensis* was approximately 23% (9), 69.6% for marandu, and 93.4% for Mombasa grass (14).

Conclusion

The forages showed satisfactory responses in growth and biomass accumulation in an irrigated culture during winter in the Goiás' Cerrado. Mombasa grass stood out among the forages of the genus *Panicum* and MG5 in *Brachiaria*. Thus, Mombasa and *Brachiaria* MG5 grasses are recommended for the cultivation of irrigated forage during winter in the Goiás' Cerrado.

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Spatial pattern of mottled stripe disease (*Herbaspirillum rubrisubalbicans*) in sugar cane

Patrón espacial del moteado clorótico (*Herbaspirillum rubrisubalbicans*) de la caña de azúcar

Rosalba Quiñones-Valdez [1], Hilda Victoria Silva-Rojas [2], Alvaro Castañeda-Vildozola [1], Omar Franco-Mora [1], Apolonio Valdez-Balero [3], Jesús Ricardo Sánchez-Pale [1*]

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Abstract

Sugar cane (*Saccharum* spp. hybrids) is the most important agroindustrial crop of the tropics. Recently, sugar cane plants with chlorotic leaf streaking associated with the bacterium *Herbaspirillum rubrisubalbicans* have been observed. This disease impacts photosynthetic capacity and yield. Characterizing the spatio-temporal behavior of chlorotic mottling using prediction maps is an important element of integrated disease management. Here, we determine the spatial distribution of mottled stripe disease in sugar cane in southeastern Mexico. To do this, we randomly chose and georeferenced 80 points in commercial plots in 2016 and 2017 to determine the disease incidence. We generated an experimental semivariogram based on a predetermined theoretical model and estimated Kriging. The incidence was 2.93% in 2016 and 5.36% in 2017 in varieties ICP-MEX-92-1420, CP-72-2086, ITV-92-373, MEX-79434 and MEX-69-290. The spatial behavior of the bacteria fit the pentaspherical model in 2016 and the spherical model in 2017. Spatial interpolation was validated by Mean Error (ME), Root Mean Square Error (RMSE) and Mean Standardized Prediction Error (MSPE) values near zero, visualized using the generated map. The results will be used to guide management of mottled stripe disease in sugar cane in the affected area.

Keywords
bacteria • incidence • spatio-temporal distribution • *Saccharum* spp.

1 Programa de Postgrado en Ciencias Agropecuarias y Recursos Naturales. Facultad de Ciencias Agrícolas. Campus Universitario “El Cerrillo” Piedras Blancas. Toluca. Estado de México. CP 50200. México.* jrsanchezp@uaemex.mx
2 Laboratorio de Biotecnología y Patología de semillas del Colegio de Postgraduados. Km 36,5 carretera México Texcoco Montecillo. Estado de México. CP 56230. México.
3 Colegio de Postgraduados Campus Tabasco. Periférico S/N Heroica Cárdenas. Tabasco. CP 86500. México.

Resumen

La caña de azúcar (*Saccharum* spp. híbridos) es el cultivo agroindustrial más importante de regiones tropicales. Recientemente, en sus hojas se han observado estrías cloróticas asociadas con la bacteria *Herbaspirillum rubrisubalbicans* que afecta la capacidad fotosintética de la planta y su rendimiento. El comportamiento espacio temporal es un elemento importante para el manejo integral de las enfermedades. Con el objetivo de determinar la distribución espacial del moteado clorótico en caña de azúcar en el sureste de México, se georreferenciaron 80 puntos en forma aleatoria en 2016 y 2017, para determinar la incidencia de la enfermedad. Se generó un semivariograma experimental ajustado a un modelo teórico preestablecido y se estimó el Kriging. La incidencia de la enfermedad fue de 2,93% (2016) y 5,36% (2017) en las variedades ICP-MEX-92-1420, CP-72-2086, ITV-92-373, MEX-79434 y MEX-69-290. El comportamiento espacial se ajustó al modelo Penta-esférico en 2016 y Esférico en 2017. La interpolación espacial se validó con valores cercanos a cero de la Media del Error (ME), Raíz del Error Cuadrático Medio (RMSE) y Media Estandarizada del Error de predicción (MSPE), visualizándose en el mapa obtenido. Los resultados permitirán direccionar el manejo integral del moteado clorótico en caña de azúcar en la zona afectada.

Palabras clave

bacteria • incidencia • distribución espacio-temporal • *Saccharum* spp.

Introduction

Sugar cane has a high capacity for biomass production because it efficiently converts energy into carbohydrates and stores sucrose in its stalk (26). Sugar cane is easy to produce, contributes food for both livestock and humans, and is an important industrial input (14, 30). Mexico is the sixth largest producer of sugar cane worldwide, contributing about 3.5% of total sugar cane production, and about 48% of the sugar cane produced in Mexico is from the southeastern region of the country, with an average yield of 74 t ha^{-1} (33). Like any monoculture, sugar cane production is affected by phytosanitary problems that decrease yield, juice purity, and the industrial quality of the product. Control measures include using more tolerant varieties and thermally or chemically disinfecting seeds (15). Despite these measures, there has been a persistent increase in the incidence of diseases, especially mottled stripe disease.

Mottled stripe disease (also known as "chlorotic spotting") causes the appearance of chlorotic spots over the surface of the leaf blade, which join together to form lines or streaks. Over time, the streaks change color to red-orange and eventually cover the entire leaf, reducing the plant's photosynthetic capacity (34). The causal organism of mottled stripe disease is *Herbaspirillum rubrisubalbicans* (=*Pseudomonas rubrisubalbicans*), which was first isolated from diseased sugar cane leaves by Hale and Wilke (1972). Baldani *et al.* (1996) later confirmed that *H. rubrisubalbicans* is an endophytic diazotroph that colonizes the roots, stalks, and especially leaves of sugar cane plants (*Saccharum* spp. Hybrids), leading to chlorotic spotting.

Chlorotic spotting is currently found in 30 countries, mainly in Africa and the Americas (5, 33). Given the diversity of varieties cultivated in different regions, some sugar cane genotypes are apparently more susceptible to the disease, which is mostly expressed in young plants (5). In Mexico, chlorotic spotting is considered a re-emerging disease; it was first reported in the country in 1996 in some sugar cane, maize, and sorghum plots (5). Initially, no crop damage was reported, but chlorotic spotting currently leads to considerable losses in southeastern Mexico (34).

More information on the location of the disease and the ability to predict its occurrence over time is necessary to prevent future infections. Geostatistics and geographical information systems have been used as tools in integrated disease management. These tools can help determine the spatial and temporal distribution of a pathogen or insect pest, estimate the percentage of infection or infestation, and make decisions on specific control measures to reduce costs (9, 31). Maps of the spatial distribution and percentages of infection/infestation can also be used to guide control measures to reduce contaminating particles and economic losses (9, 23).

Spatial modeling and digital mapping use computational algorithms and predictors that represent the variables to map (11, 24). In addition to their use in the management of diseases from a site-specific perspective, these models have research applications in biology, the potential impacts of climate change, epidemiology, biogeography, and geographic regions that require study (25). In recent years, understanding the spatial distribution of diseases within the production area has been considered essential for efficient disease management. This contributes to the development of a sustainable production system by focusing on control options that decrease damage, reduce costs, and yield a high-quality product (12). In this study, we aimed to determine the spatial patterns of incidence of mottled stripe disease in sugar cane in southeastern Mexico.

Materials and methods

The study area included the commercial sugar cane production area between the coordinates 18°23’55” North and 95°46’47” West in the gulf region of southeastern Mexico. In the years 2016 and 2017, we sampled plants with symptoms of mottled stripe disease ten months after harvest (plant crop, first stubble crop or second stubble crop). For each sampling, we considered 80 points that were randomly generated by the program QGis. Each point was located in the study area using a Global Positioning System (model Garmin-GPSmap USA) and georeferenced in the field. At each point, the percent incidence of mottled stripe was determined over 10 linear m using the formula: $\% I = \Sigma PA/\Sigma PT \cdot 100$, where: % I is the percent incidence of disease symptoms, PA is the number of plants with symptoms of mottled stripe, and PT is the total number of plants sampled along 10 linear m from the georeferenced point. Chemical control measures were not applied in any of the plots sampled. Monthly minimum and maximum temperature and precipitation were obtained from the Mexican agencies (8) for the 2015-2016 and 2016-2017 growing seasons.

Leaves exhibiting typical chlorotic mottling symptoms were collected at each point to determine the causal agent using molecular testing at the Seed Pathology Laboratory at the College of Postgraduates, Montecillo Campus in Texcoco, Mexico State. The sampled leaves were disinfected externally with a 1% (v/v) sodium hypochlorite solution for 1 min, rinsed three times with sterile distilled water, then placed on absorbent paper to remove excess moisture. Leaf tissue sections measuring 0.5 × 0.5 mm were transferred to Petri dishes containing King’s B medium then incubated at 28°C for 48 h. Whitish-colored colonies developed, which were then cultured to obtain pure cultures. Bacterial isolates underwent molecular characterization using DNA extracted from the pure cultures of each isolate under the cetyltrimethylammonium bromide (2%) buffer (CTAB) protocol (13). The DNA was quantified by spectrophotometry in a Nanodrop 2000 (Thermo Scientific, USA) and considered to have adequate quality for PCR analyses when the $A_{260/280}$ and $A_{260/230}$ ratios were between 1.8 and 2.2.

The 16S rDNA was amplified *via* PCR using the universal primers 8F (5-AGAGTTT-GATCCTGGCTCAG-3’) and 1492R (5´-GGTTACCTTGTTACGACTT-3’). The PCR reactions were carried out in a total volume of 15 μL containing 100 ng DNA, 0.8 mM of dNTP, 0.3 U of Go *Taq* DNA polymerase (Invitrogen Carlsbad, CA, USA), 5x Go *Taq* reaction buffer (Promega, Fitchburg, WI, USA), and 10 pmol of each primer. The PCR parameters were as follows: single preheating step at 95°C for 2 min; 35 cycles of 95°C for 2 min, 59°C for 1 min, and 72°C for 1.5 min; and a final extension at 72°C for 5 min (26). All PCR reactions were done in a DNA Engine thermal cycler (BioRad, CA, USA), and the PCR products were verified by loading 5 μL into a 1.5% agarose gel in 1x Tris Acetate-EDTA buffer electrophoresed at 111 Volts for 1 h. The amplicons were visualized using an Infinity-3026 WL/LC/ 26MX transilluminator (Vilber Lourmat, Germany). The remaining volume of PCR product was cleaned with the Exosap-IT enzyme protocol (Affymetrix, USA) following the manufacturer’s instructions. To ensure that there were no misreadings, the PCR products were sequenced in both directions with primers 514F (5’- GTGCCAGCMGCCGCGG-3’) and 800R (CTACCAGGGTATCTAAT-3) in a genetic analyzer (Applied Biosystems, CA, USA), using a Big Dye Terminator V.3.1 Cycle Sequencing kit standard (Applied Biosystem, CA, USA).

The sequences corresponding to both strands of the 16S rDNA were assembled and edited using BioEdit v7.0.5 (19), generating a consensus sequence of each isolate. Since the basic local alignment search tool from NCBI finds regions of local similarity between sequences with significant alignments, the consensus sequences of each isolate were submitted to BLASTN 2.10.0 (3). Sequences obtained in this study were deposited in the GenBank database.

The geostatistical analysis of the incidence data consisted of estimating a semivariogram and the parameters of the model as well as generating maps using Kriging interpolation. The incidence data were transformed prior to analysis using the inverse-logarithm to homogenize variance. The experimental semivariogram value was calculated based on the equations established by Goovaerts (1999) and Isaaks and Srivastava (1989):

$$\gamma^*(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(H)} [z(x_i + h) - z(x_i)]^2$$

where
$y^*(h)$ = the experimental value of the semivariogram for the distance interval h;
$N(h)$ = the number of pairs of samples separated by distance interval h;
$z(x_i)$ = the value of the variable of interest at the sampling point x_i;
$z(x_i+h)$ = the value of interest at the sampling point x_i+h.

The experimental semivariogram was fit to a predetermined theoretical semivariogram. The degree of spatial dependence was calculated to determine the relationships among the data. The experimental semivariogram and the values from the theoretical model were obtained using the program R (7, 29), using the gstat library (24).

Seven theoretical models (spherical, exponential, gaussian, linear, matern, bessel, and pentaspherical) were tested to select the one that best fit the experimental semivariogram of disease incidence. The best model was the one with the lowest error (9, 10) based on the following formula

$$\mathrm{SSE} = \sum_{i=1}^{m} w_i \, [\tilde{y} - y]^2$$

where
m = the lag number (points separated by a given distance);
$\tilde{y}$ = the semivariance values for each distance; y is the semivariance values from the prediction model;
w_i = the semivariance factors obtained from the equation: $w_i = N/\tilde{y}^2$

where
N = the number of pairs of points used to calculate $\tilde{y}$ for each distance (9, 10).

Kriging (27) was then done using the model with the lowest error. Three types of kriging were evaluated (simple, ordinary, and universal) using the cross-validation technique. For interpolation, the kriging with the lowest precision error was selected based on the lowest mean error (11); root mean square error (1, 23, 35) and mean standardized prediction error (11), which should all have values close to zero. The result of kriging was a spatial layer in Geotiff format generated using the rgdal package for R. Finally, the map was edited using the program Surfer 15 (Surface Mapping System, Golden Software Inc. 809, 14th Street Golden, CO, 804011866, USA) to visualize the spatial pattern of *H. rubrisubalbicans*.

Results

The average incidence of chlorotic mottling symptoms was 2.93% in the 2016 season and 5.36% in 2017 (table 1, page 130). Molecular characterization (5, 34) confirmed that the bacterium *Herbaspirillum rubrisubalbicans* was present in all the samples tested.

Table 1. Incidence of mottled stripe (*Herbaspirillum rubrisubalbicans*) in different varieties of sugar cane in commercial plots in southeastern Mexico in the 2016 and 2017 seasons.

Tabla 1. Incidencia del moteado clorótico (*Herbaspirillum rubrisubalbicans*) en diferentes variedades de caña de azúcar de parcelas comeciales del sureste de México durante los ciclos 2016 y 2017.

No.	Variety	Incidence (%)	
		2016	2017
1	ICP-MEX-92-1420	75	29
2	COLMEX-95-27	36.66	0
3	CP-72-2086	31.66	60
4	CP-69-290	15	0
5	ITV-92-373	11	1
6	MEX-79-431	10	13
7	MEX-69-290	6.66	30
9	MEX-68-P-23	1.66	0
10	RD-75-11	0	1
11	SP-70-1284	0	0
12	ITACMEX-57-197	0	0
13	Mayan-55-14	0	0
14	SP-79-2233	0	0
15	ICP-74-2005	0	0
17	MEX-68-P-23	0	0
18	MEX-69-1420	0	0

The mean maximum temperature was 30°C, the mean annual temperature was 23.5°C, and mean annual precipitation was 127.8 mm in 2016, while in 2017 the mean annual precipitation was 144.3 mm and the mean annual temperature was 23.2°C (figure 1) (8). Higher precipitation and temperature were associated with a higher percent incidence of mottled stripe in 2017.

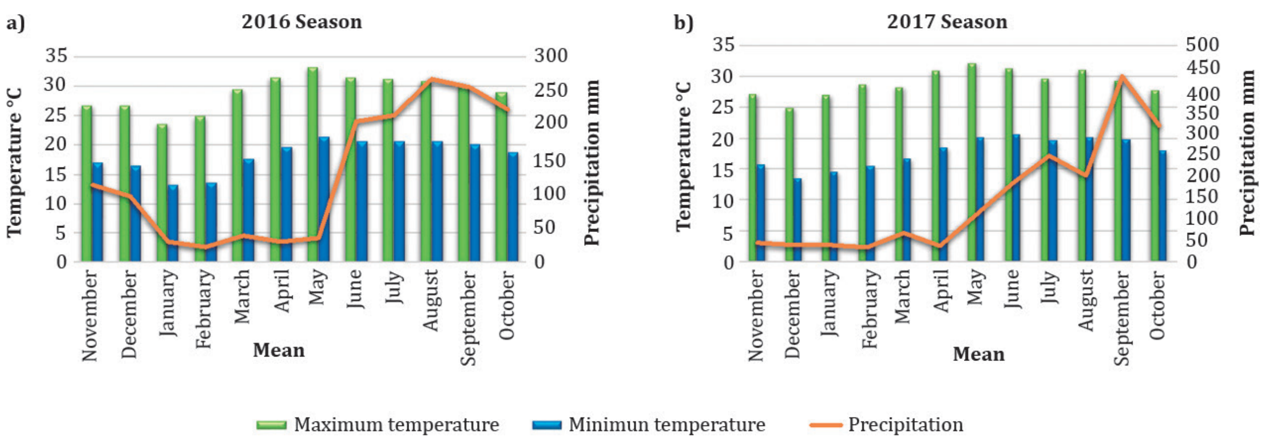

Figure 1. Precipitation (right axis) and minimum and maximum monthly temperatures (left axis) from November 2015 through October 2016 (2016 season; a) and from November 2016 through October 2017 (2017 season; b), in southeastern Mexico.

Figura 1. Precipitación y temperaturas máximas y mínimas mensuales durante el ciclo de noviembre de 2015 a octubre de 2016 (a) y de noviembre de 2016 a octubre 2017 (b) en el sureste de México.

The overall incidence of mottled stripe increased by 2.7% from 2016 to 2017. The ICP-MEX-92-1420 variety had the highest percent incidence, followed by COLMEX-95-27 and CP-72-2086, which had the largest affected area. The CP-72-2086 variety was the most preferred by producers and had the largest cultivation area in the region. In 2017, the mottled stripe incidence in this genotype doubled. The COLMEX-9527 variety had the second highest incidence in 2016, and in the MEX 69-290 variety, the incidence quadrupled from 2016 to 2017 (table 1, page 130).

The geostatistical analysis showed that the disease had an aggregate distribution in both years of the study. The pentaspherical model was the model that best fit the data on disease incidence in 2016 (figure 1a, page 130), while in 2017 (figure 1b, page 130) the incidence data were better described by the spherical model. This shows a pattern of behavior with strongly defined points in both years. In 2016, the disease was expressed in more of the varieties but at a lower percent incidence. In 2017, fewer of the varieties were affected by the bacteria, but there was an increase in the percent incidence; in other words, the disease was apparently more aggressive.

In the semivariogram for 2016, there was spatial autocorrelation of the presence of mottled stripe in the sugar cane crop at distances up to 1535.68 m (range), beyond which the correlation among points decreased. This suggests that future sampling should consider this distance between points for regional-level studies. In addition, there was high degree of spatial dependence. In 2017, the range of correlation was 1296.37 m, with moderate spatial structure or degree of spatial dependence (table 2).

Table 2. Incidence and parameters from the fitted semivariogram models based on sampling of mottled stripe disease in sugar cane (*Herbaspirillum rubrisubalbicans*) in commercial plots in southeastern Mexico during the 2016 and 2017 growing seasons.

Tabla 2. Incidencia y parámetros de los semivariogramas ajustados a modelos del moteado clorótico de la caña de azúcar (*Herbaspirillum rubrisubalbicans*) en plantaciones comerciales del sureste de México, durante los ciclos 2016 y 2017.

Season	Incidence %	Model	Nugget	Sill	Range	Nugget/Sill (%)	Degree of dependence	Kriging	Cross-validation		
									ME	RMSE	MSPE
2016	2.93	Pentaspherical	0.003	0.884	1535.68	0.339	High	Ordinary	0.032	0.996	0.993
2017	5.36	Spherical	0.655	1.181	1296.37	55.46	Moderate	Ordinary	0.027	1.380	1.906

The maps of the presence of the disease in southeastern Mexico and its pattern of behavior (figure 2, page 132) indicate that in 2016 the bacterium was distributed in practically the entire region, except for the northeast, while in 2017 the disease was less dispersed, with a more focused presence in the southeast and northeast of the map of the study region. In 2016, the bacterium was found from the central zone through the southern part of the sugar cane region, while in 2017 it was distributed in the northeastern region of the study area.

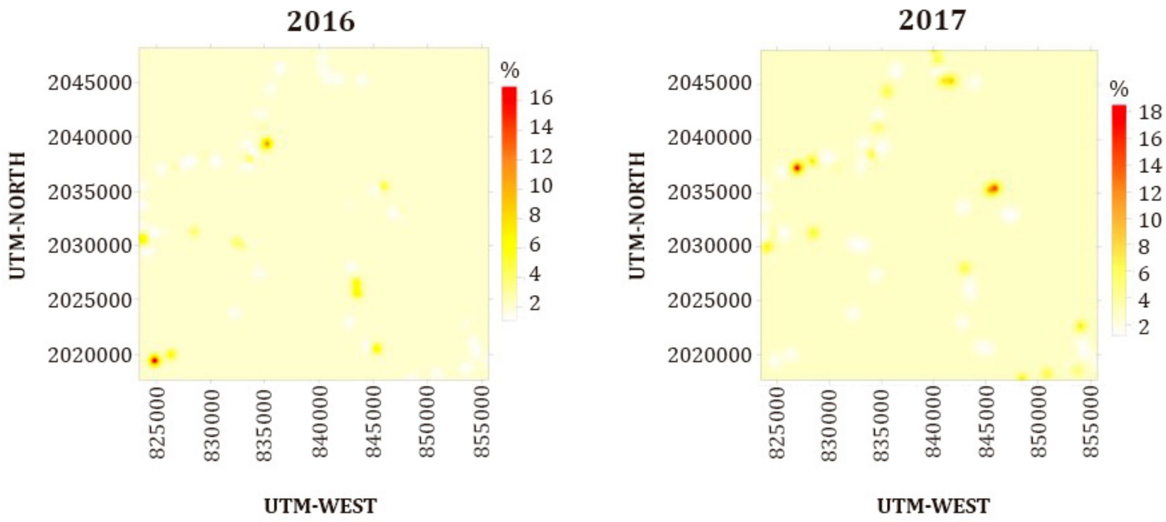

Figure 2. Spatial distribution maps of incidence of symptoms of mottled stripe caused by *Herbaspirillum rubrisubalbicans* in southeastern Mexico in 2016 (a) and 2017 (b).

Figura 2. Mapas de la distribución espacial de la incidencia del moteado clorótico causada por *Herbaspirillum rubrisubalbicans* en el sureste de México durante 2016 (a) y 2017 (b).

Discussion

The bacterium *H. rubrisubalbicans* was present throughout the study region and during both years of the study (2016-2017), indicating that this pathogen can persist from one growing season to the next and spread from one plot to another by different mechanisms (2). It was also apparently well adapted to the climatic conditions in this sugar cane producing region, as well as the ICP-MEX-92-1420, CP-72-2086, ITV-92-373, MEX-79-434, and MEX-69-290 genotypes, which had the highest incidence of mottled stripe during both years of the study. The average temperature was similar between the two years of the study, but there was higher average annual precipitation in 2017, which was associated with a higher percent incidence of the bacterium *H. rubrisubalbicans*. In other words, when precipitation increased there was increased spread of the bacteria.

The spatial patterns expressed by diseases of bacterial origin can be influenced by a variety of factors, including climate, soil characteristics, and progression of the epidemic throughout the zone (22), as well as sampling protocols (6). Patterns of bacterial infection may also be influenced by host susceptibility (17). This is consistent with findings of different spatial patterns among different varieties of sugar cane that have different levels of susceptibility by Belan *et al.* (2018) and in the 2017 season in this study. The differences in spatial patterns between the 2016 and 2017 seasons show the dispersion potential of the disease in this crop system, as well as a latent infestation in the area, which means that the disease may be expressed whenever more susceptible genotypes are planted (6). In addition, the increase in incidence could have been due to means of dispersal such as the sowing or propagation of infected stalks, working with contaminated equipment or tools, or growing more susceptible cultivars or genotypes that are less tolerant to the pathogen.

Though we lack concrete data on the economic losses cause by the bacterium, it is evident that the leaf lesions affect the photosynthetic capacity and development of the plant. (5) indicate that the strain NCPPB 1027 (= LMG 2286) of *H. rubrisubalbicans* is considered a mild plant pathogen, which is found in sugar cane crops and only affects susceptible cultivars. However, our results show that the incidence, and thus potential damage to production, increases substantially from one cycle to the next, reducing the quality of sugar cane production in this region.

The semivariograms and maps show aggregate behavior of this disease, expressed as different spatial patterns given by the spatial dependence among points and the severity of the effects in each plot. In this sense, Contreras-Rendón *et al*. (2014) report spatially aggregated behavior of the bacterium *Candidatus* Liberibacter solanacearum, as has also been reported for *Xanthomonas arboricola* pv. corylina (22) in hazelnuts and *Xanthomonas campestris* pv. musasearum (32) in banana.

The differences in the spatial patterns found in the distribution of *H. rubrisubalbicans*, both in physical space in the region and between the two years of the study are similar to those reported by Contreras-Rendón *et al*. (2014) for *Candidatus* Liberibacter solanacearum in potato crops. The spatial distribution fit the pentaspherical model in 2016, which indicates that the aggregations are found in specific points but at low incidences in eight genotypes. On the other hand, the spatial distribution in 2017 was best fit by the spherical model, indicating the existence of focalized aggregations (4) with a higher percent incidence. Our maps based on the spatial distribution of mottled stripe over a continuous space help detect the areas that are most severely affected and require immediate control measures (29). These maps can also be useful to associate particular environmental characteristics with patterns of disease distribution, which can point to possible preferences in the aggregation structure of the bacteria in the study region (21). Here, changes in the spatial distribution of the disease from one year to the next and the greater number of new outbreaks of the bacteria in 2017 are likely due to the presence of susceptible genotypes and a favorable environment.

Conclusion

The chlorotic mottling of sugar cane showed an aggregate type spatial behavior at the regional level. There was some difference in spatial patterns between years, which corresponded with an increase in precipitation and more susceptible sugar cane genotypes. However, the patterns in both years showed consistently focalized points.

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