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Índice

Influence of genotype-environment interaction on soybean (*Glycine max* L.) genetic divergence under semiarid conditions

Influencia de la interacción genotípica por ambientes en la divergencia genética de la soja (*Glycine max* L.) en condiciones semiáridas

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Abstract

The objective of the present work was to evaluate the influence of the genotype–environment interaction on genetically divergent soybean grown under semiarid conditions. Four experiments were carried out in randomized blocks with four replicates to evaluate twenty-one soybean genotypes. The following descriptors were used to quantify divergence: plant height, dry matter, oil content, number of pods per plant, number of grains per pod, the weight of 100 seeds, yield, days for flowering, and days for maturation. The unweighted pair group method with arithmetic mean was used to group the genotypes from the Mahalanobis distance matrix estimated using the genotypic means estimated by the REML/BLUP method. The grouping of genotypes depended mainly on the effects of the interaction between genotypes and years. The joint analysis, without the effect of the interaction, allowed us to obtain two groups of genotypes. The most recommended crosses were those of the lines BRS Tracajá, BRS Pérola, BRS Carnaúba, M 8644 IPRO, BRS 8590, and BMX OPUS IPRO with the genotype BRS Sambaíba, especially the one between BMX OPUS IPRO and BRS Sambaíba.

Keywords
Glycine max L.• germplasm • dissimilarity • choice of parents.

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Resumen

El objetivo del presente trabajo fue evaluar la influencia de la interacción genotipo por ambiente en estudios de divergencia genética en soja en condiciones semiáridas. Se realizaron cuatro experimentos con un diseño de bloques completos aleatorizados con cuatro repeticiones donde se evaluaron 21 genotipos de soja. Se utilizaron los siguientes descriptores para cuantificar la divergencia: altura de planta, materia seca, contenido de aceite, número de vainas por planta, número de granos por vaina, peso de cien semillas, rendimiento, días a floración y días a maduración. Se utilizó el método jerárquico UPGMA para agrupar los genotipos de la matriz de distancias de Mahalanobis mediante las medias genotípicas estimadas por el método REML / BLUP. La agrupación de genotipos depende de las condiciones de evaluación, principalmente debido a los efectos de la interacción genotipo por años. El análisis conjunto, sin la presencia del efecto de interacción, permite obtener dos grupos de genotipos. Los cruzamientos más adecuados involucran los genotipos BRS Tracajá, BRS Pérola, BRS Carnaúba, M 8644 IPRO, BRS 8590 y BMX OPUS IPRO, y el genotipo BRS Sambaíba, especialmente el entre BMX OPUS IPRO y el genotipo BRS Sambaíba, que es el de mayor disimilitud.

Palabras claves

Glycine max L. • germoplasma • disimilitud • elección de padres

Introduction

Soybean (*Glycine max* L. Merrill) is a legume of great importance in the world economy because of its extensive cultivation and world trade. It is widely used as a raw material in animal feed, food, vegetable oils, biofuel, and the chemical industry. Brazil is the largest exporter and the second-largest producer of oilseeds. In the 2019/2020 harvest, the country had an average productivity of 3.321 kg ha^{-1}, resulting from a production of 118.8 million tons of grain in 35,760.4 thousand hectares of land. During this harvest, the cultivated area increased by 1.7% relative to the past harvest (2, 10), due to the expansion of the agricultural frontier with the exploration of the Brazilian semiarid region. Considering the need for irrigation, as well as the high temperatures that prevail in the semi-arid region, this expansion may constitute an opportunity for the diversification of crops in the region, particularly for seed production since irrigated crops require greater investment. Therefore, it is necessary to work with cultivars adapted to the new growing conditions, which requires the evaluation of the behavior of soybean genotypes under the non-edapho-climatic and specific desired agricultural conditions. It is crucial to consider the possibility of not finding an adapted genotype with the desired characteristics among those available. In this case, it is necessary to work with breeders to develop adapted cultivars with characteristics suitable for the market.

The cultivar experiments, sometimes called value of cultivation and use (VCU) tests, are generally carried out in different environments, either local or through years of cultivation, characteristic of the region of interest. When several genotypes are evaluated in more than one environment, the presence of a genotype-environment interaction is common (G × E). This phenomenon is defined as the differential behavior of genotypes across environments and assumes an important role in phenotypic manifestation. The G × E interaction, when predominantly qualitative or crossed, hinders the selection process or recommendation of cultivars, since the order of genotypes is altered in the assessment environments.

Information on genotypic performance may also be relevant when choosing the parent strains for breeding programs. In this context, although productivity is the characteristic of greatest interest, other characteristics are relevant for soybeans and are therefore considered in the genotypic evaluation. When information on various traits-whether morphological, agronomic, biochemical, or molecular-is available, it becomes possible to carry out genetic divergence studies. However, even though the presence of genotype-environment interaction in soybeans and other crops is notorious, there is little information about its influence on divergence studies since genetic divergence studies are generally performed in only one evaluation environment.

Filling this knowledge gap requires intensive efforts of researchers, more specifically the curators of germplasm banks and breeders since divergence studies are important with respect to two main aspects. The first aspect is related to the intrinsic activities carried out in germplasm banks to evaluate the entire variation structure to preserve it and optimize its maintenance in nuclear collections. A second aspect, no less important in relation to the first, is that studies of genetic divergence are used to define groups with the intention of directing crossings that could potentially generate segregated populations with greater genetic variability (25, 34). Indeed, considering that the interaction between genotypes and environments can alter the characterization of genotypes in groups according to environmental conditions, it may be challenging to define the crossings.

The aforementioned considerations and the lack of studies of this nature motivated the realization of the present work, which proposes to study the effect of the genotype–environment interaction on the genetic divergence of soybean genotypes evaluated under semiarid conditions.

Material and methods

Environmental characterization

This study was carried out during the dry seasons of 2016-2017, and rainy seasons of 2017-2018 in the municipality of Mossoró, Rio Grande do Norte, Brazil (5° 03' 37″ " S, 37° 23' 50"″ W, 72 m altitude). According to the Koppen climate classification (1), the climate of the site is DdAa, semi-arid, and megathermal, with little or no excess precipitation during the year; in addition, it is BShw – dry and very hot.

The average meteorological data for the experimental period are shown in figure 1 (page 4). The experiments were started on the following planting dates: September 25, 2016, March 29, 2017, September 30, 2017, and March 16, 2018.

The soil of the experimental field was classified as typical red dystrophic argisol (14), whose chemical analysis results, at a depth of 0.20 m, before starting each experiment are shown in table 1.

Table 1. Chemical analysis of the soil, in each year of cultivation, referring to the depth of 0.20 m.

Tabla 1. Análisis químicos del suelo, para cada campaña, referidos a la profundidad de 0,20 m.

Crops	N (g kg^{-1})	OM** (g kg^{-1})	K	P	Na	Ca	Mg	pH	EC** ds m^{-1}
			-------mg dm^{-3}--------			cmol$_c$ dm^{-3}			
2016/17	0.15	8.03	54.03	4.23	8.30	2.30	1.20	6.64	0.56
2017.1	0.42	12.95	41.71	2.17	8.61	1.05	0.93	6.32	0.67
2017.2	0.35	11.78	53.73	3.50	4.20	1.00	1.12	5.87	0.73
2018.1	0.41	10.53	27.12	2.34	8.54	1.56	1.24	6.20	0.47

**EC = electrical conductivity; OM = organic matter.

**CE = Conductividad eléctrica; MO = materia orgánica.

Germplasm

The identification and characteristics of the soybean genotypes evaluated and belonging to Embrapa Meio-Norte are shown in table 2 (page 5).

Experimental details

The seeds were inoculated before sowing with 4 g of peat inoculant per kg of seeds (TotalNitro Ultra) and 500 ml ha^{-1} of liquid (TotalNitro Full) was applied to the seeds in the planting furrow, ensuring that the seeds were completely covered by the inoculant. The inoculants were obtained from Total Biotecnologia, Curitiba, Paraná, Brazil. According to the desired spacing, sowing was performed manually with the help of previously drilled wooden rulers.

Weed control was performed manually with hoes after sowing, as recommended (Dugje *et al.* 2009). Due to the unstable precipitation during the experimental period, water complementation was carried out when necessary. Irrigation was carried out by spraying, with a daily watering shift divided into two applications (morning and afternoon), according to the water requirement of the culture and based on the reference evapotranspiration (ETo) rate. During the rainy season, irrigation was performed only when necessary.

Fertilization was carried out as recommended for the crop and based on the results of the soil analysis, with 60 kg ha^{-1} of P_2O_5 being applied during planting and 60 kg ha^{-1} of K_2O in coverage (16). The sources of Phosphorus and Potassium were simple superphosphate and potassium chloride, respectively.

Figure 1. Mean values of maximum, minimum and average air temperature (°C), relative humidity (%), solar radiation (MJ m^{-2} day^{-1}) and rainfall (mm) for four soybean crops in the years 2016/2017, 2017.1, 2017.2 and 2018.1.

Figura 1. Valores promedio de temperatura máxima, media y mínima del aire (°C), humedad relativa (%), radiación solar (MJ m^{-2} día^{-1}) y precipitación (mm) para cuatro cultivos de soja en las campañas 2016/2017, 2017.1, 2017.2 y 2018.1.

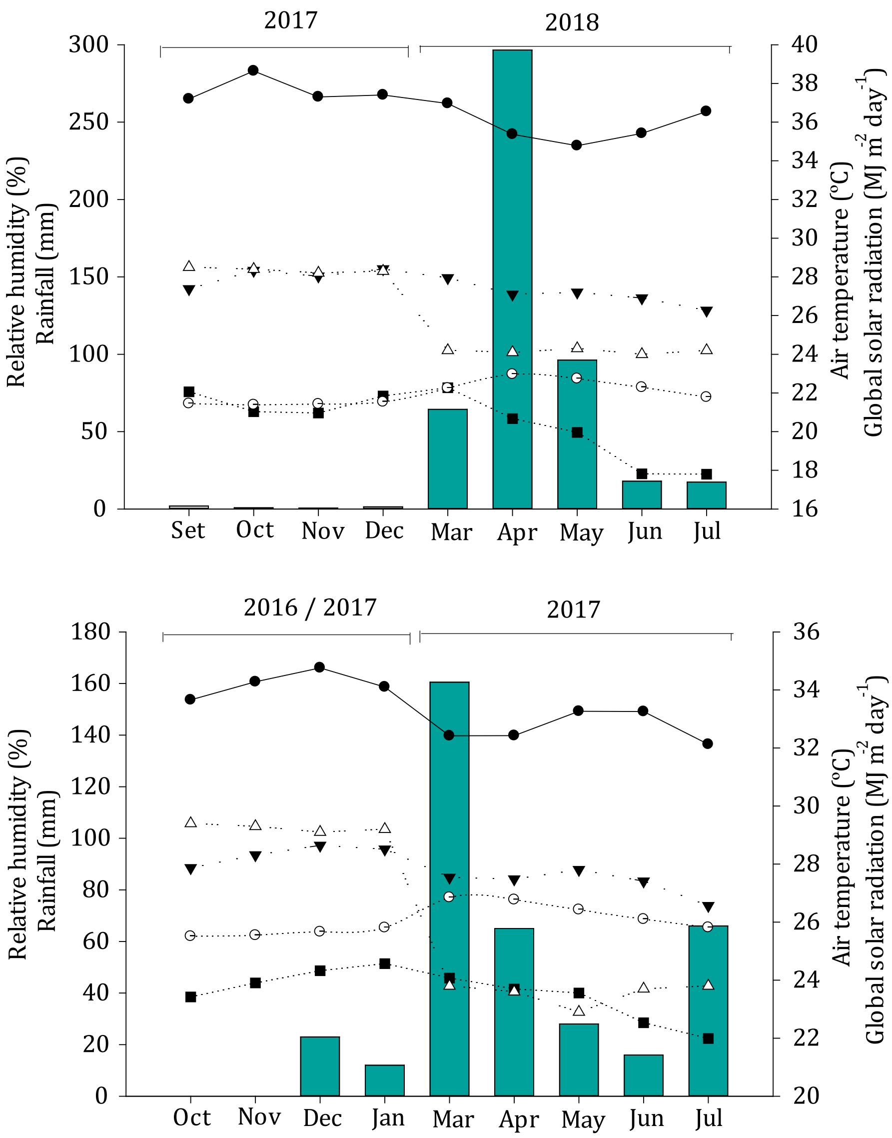

Table 2. Category, identification, name, maturation group and cycle of 21 soybean genotypes evaluated in four harvests under semiarid conditions.

Tabla 2. Categoría, identificación, nombre, grupo de madurez y ciclo de 21 genotipos de soja evaluados en cuatro cosechas en condiciones semiáridas.

Category	Identification	Name	Group (Maturation)	Cycle (days)
1	G02	'BRS Carnaúba'	9.6	101
	G03	'BRS Pérola'	8.8	103
	G04	'BRS Tracajá'	9.2	101
	G05	'BRS Sambaíba'	9.3	100
	G07	'BRS 8590'	8.5	104
2	G01	'BMX OPUS IPRO'	8.6	101
	G08	'BRS 9383 IPRO'	9.3	103
	G09	'BRS 9180 IPRO'	9.1	111
	G12	'M 8644 IPRO'	8.6	102
	G13	'M 8372 IPRO'	8.3	106
3	G06	'BRS Sambaíba RR'	9.3	107
	G10	'BRS 333 RR'	9.4	102
	G11	'BRS 9280 RR'	9.2	105
	G14	'P 98Y70 RR'	8.7	102
	G15	'ST 920 RR'	9.2	106
	G16	'Pampeana 10 RR'	9.8	111
	G17	'Pampeana 20 RR'	10.0	118
	G18	'Pampeana 40 RR'	9.5	111
	G19	'Pampeana 50 RR'	9.6	111
	G20	'PAS 13565-74 RR'	9.5	117
	G21	'Pampeana 007 RR'	9.7	111

Category 1: conventional soybean genotypes. Category 2: high-yield soybean genotypes, resistant to glyphosate and which have protection and suppression against some soy pests. Category 3: glyphosate resistant soybean genotypes.

Categoría 1: genotipos convencionales de soja. Categoría 2: genotipos de soja de alto rendimiento, resistentes al glifosato y que tienen protección y supresión contra algunas plagas de la soja. Categoría 3: genotipos de soja resistentes al glifosato.

Evaluated keywords

The evaluated characteristics were: a) plant height: measured using a ruler, across ten random plants in the plot in phase R5.3, according to the methods described in the study of (9, 35); b) dry matter: evaluated using samples from ten plants per plot; the samples were placed in an oven with forced air circulation at 65 °C, until they reached a constant weight, according to the method of Brandt *et al.* (2006); c) oil content: determined with 30 g (ground) of seeds from each plot using the Near Infrared Reflection (NIR) technique and expressed as a percentage (%), according to the method of Heil (2010); d) number of pods per plant: obtained by counting the number of pods per plant; e) number of grains per pod: obtained by counting the number of grains in the pods divided by the total number of pods; f) weight of one hundred seeds: determined according to the mass and total number of seeds per experimental unit; g) productivity: upon reaching physiological maturity (95% of the mature pods), the soybean plants were harvested from the two central rows of each plot, 4 m^2, with a 0.5 m edge left unharvested. After harvesting, the plants were trailed, and the seeds were then weighed, after drying (12% humidity) and cleaning, to determine the grain yield of kg ha^{-1}; (h) days for flowering: the days from emergence necessary to have an open flower in 50% of the plants of the plot; this characteristic was evaluated using ten plants chosen at random, according to the method employed by Carvalho (2014); and i) days for maturation: determined from the number of days from emergence necessary to have 95% of mature pods; this characteristic was evaluated using ten random plants in the plot, according to that method.

Statistical analysis

The REML/BLUP analysis was performed using Model 54 of the SELEGEN software (30). Through this model, the empirical BLUP predictors of genotypic values free from interaction were obtained, and were given by $\hat{\mu} + \hat{g}_i$, where $\hat{\mu}$ is the average of all environments, and $\hat{g}_i$ is the free genotype effect of the genotype × environment interaction. For each environment "$_j$" genotype values are predicted by $\hat{\mu} + \hat{g}_i + \widehat{ge}_{ij}$, where $\hat{\mu}_j$ is the environment average "$_j$"

$\hat{g}_i$ is the genotypic effect, and $\widehat{ge}_{ij}$ is the effect of the genotype × environment interaction concerning the genotype "$_i$". The generalized Mahalanobis distances were calculated from the matrix of the genotypic averages of each characteristic for each genotype and from the residual variance-covariance matrix (20). Cluster analysis was performed with the Mahalanobis distance matrix between genotypes, using the unweighted pair group method with arithmetic mean (UPGMA). The quality of the adjustment was quantified using the cophenetic correlation coefficient (32). The analyses were processed using the Genes program (2016), and the Pheatmap (18) and *Biotools* (31) packages of Project R (27).

Results and discussion

The results of our experiments showed that the data obtained for the various characteristics were within the range observed for soybeans, with the lowest value observed for oil content (4.28%), and the highest for dry matter (51.18%). With the exception of dry matter, the estimated values of the evaluated characteristics were within the CV range observed for soybean (7, 11, 19, 21, 26). The highest selective accuracy was verified for flowering and maturation and the lowest for dry matter, conforming to the lowest precision observed for the latter characteristic. The accuracy of the results of the present study is considered low for dry matter (<0.30), moderate for the number of pods per plant (0.50 to 0.69), very high for flowering and maturation (>90), and high for the other characteristics (0.70 to 0.89), according to Resende and Duarte, 2007 (table 3).

Table 3. Deviance analysis, estimates of variance components and genetic and phenotypic parameters for nine characters measured in soybean genotypes evaluated in four trials conducted under semiarid conditions.

Tabla 3. Análisis de varianza, estimaciones de componentes de varianza, parámetros genéticos y fenotípicos para nueve caracteres medidos en genotipos de soja evaluados en cuatro ensayos realizados en condiciones semiáridas.

Effect	Character								
	PH	DM	OC	NPP	NGP	WHS	PR	NDF	NDM
	(Deviance) (LRT test - Chi-square)								
G	4.82*	1.82^{ns}	4.82**	4.19*	6.68**	8.93**	3.99*	6.75**	8.12**
GE	3.91*	4.71*	373.71**	159.68**	10.24**	294.67**	237.89**	0.23^{ns}	0.03^{ns}
REML	**Estimates of Variance Components and Genetic Parameters**								
$_GV$	19.31	0.39	0.81	22.59	0.02	2.09	147.74	7.83	20.91
V_{GE}	10.91	9.13	2.76	91.85	0.14	4.49	551.81	0.11	0.23
$_EV$	60.47	285.41	0.38	44.27	0.03	0.93	157.79	26.01	54.38
h^2_{gm}	0.75	0.02	0.53	0.47	0.59	0.64	0.50	0.83	0.86
$_SA$	0.86	0.14	0.73	0.68	0.77	0.80	0.71	0.91	0.93
G_c	0.64	0.04	0.23	0.20	0.44	0.32	0.21	0.99	0.99
$_EVC$	18.93	51.18	4.28	9.17	7.90	10.37	16.47	14.45	7.41

$_GV$: genotypic variance; V_{GE}: variance of genotype interaction by environments (GE); $_EV$: residual variance; h^2_{gm}: heritability of the genotype mean; $_SA$: selective accuracy; G_c: genetic correlation between all environments; $_EVC$: residual coefficient of variation; PH: plant height; DM: dry matter, in %; OC: oil content, in %; NPP: number of pods per plant; NGP: number of grains per pod; WHS: weight of one hundred seeds; PR: productivity, in kg ha^{-1}; NDF: number of days to flower; and NDM: number of days to mature.

V_G: varianza genotípica; V_{GA}: varianza de la interacción del genotipo por ambientes (GA); V_E: varianza residual; h^2_{mg}: heredabilidad del genotipo promedio; A_s: precisión selectiva; r_G: correlación genética entre todos los ambientes; CV_E: coeficiente de variación residual; AP: altura de la planta; MS: materia seca, en %; CA: contenido de aceite, en %; NVP: número de vainas por planta; NGV: número de granos por vaina; PCS: peso de cien semillas; PR: productividad, en kg ha^{-1}; NDF: número de días para florecer; y NDM: número de días para madurar.

A significant effect of genotypes was observed for all characteristics evaluated, except for dry matter, indicating genetic heterogeneity between the genotypes, a fact that corroborates the estimates of average heritability. Although not very high, this variability can be corroborated by heritability estimates, except dry matter. Heritability in the broad sense quantifies the fraction of phenotypic variance resulting from genotypic causes. Heritability ranges from

0 to 1.0, with estimates close to the unit indicating lesser environmental effect on the characteristic. Quantitative characteristics have a greater environmental effect and tend to have lower heritability values (4). This occurred with dry matter, but for most of the other characteristics, heritability can be considered intermediate. For plant height, flowering, and maturation, heritability was high, indicating less environmental effects on these characteristics.

The genotype–environment (G × E) interaction was significant for all characteristics, with the exception of the number of days for flowering and maturation (table 3, page 6). The presence of the G × E interaction is a common phenomenon in the evaluation tests of soybean cultivars and reflects the differential behavior of the genotypes in different environments (28). The variance component of the G × E interaction was superior to that of the genotypic variance for the characteristics of dry matter, oil content, number of pods per plant, number of grains per pod, weight of 100 seeds, and productivity (table 3, page 6), indicating a greater influence of the interaction on phenotypic variation. This is not commonly observed duirng the evaluation of soybean genotypes in various environments. Generally, for soybeans, the effect of genotypes has a greater effect on the phenotype (3, 5, 22, 26, 33).

The interaction can be quantitative because of the magnitude of the differences between the genotypes in the environments or qualitative due to the lack of genotypic correlation in the environment (15, 24). In this work, considering the values of genotypic correlation in all environments, ranged from 0.02 (dry matter) to 0.64 (plant height), the interaction was predominantly qualitative for all characteristics for which the G x E interaction existed (table 3, page 6). The predominance of cross-interaction hinders the work of breeders because the characteristics of the genotypes are significantly altered in different environments. In this situation, it is difficult to recommend a genotype suitable for all environments, and specific recommendations are necessary. On the other hand, it is important to emphasize that the northeast region of Brazil has proven to be highly suitable for irrigated cultivation, including that of fruits, vegetables, and grains. On the other hand, because it does not have well-defined climatic seasons, being most often defined only in terms of the rainy season and the dry season, it can lead researchers and producers to make errors when recommending genotypes for cultivation in semi-arid conditions. Thus, studies such as the one presented in this work become quite relevant since the agricultural frontier for soybean cultivation has increased in the northeast region of Brazil, as has the demand for adapted cultivars. The presence of cross-interaction has also been mentioned by several authors when evaluating soybean cultivars under different edaphoclimatic conditions (8, 11, 23, 26, 33).

The hierarchical clustering analysis (UPGMA) showed different results throughout the evaluations, although there was little discrimination between the genotypes (figure 2, page 8). It is noteworthy that the estimates of cophenetic correlation were higher than 0.85 in all evaluations, indicating a high quality of grouping; that is, there is a lot of similarity between the original and final dissimilarity matrices (28, dutra).

In 2016, three groups of genotypes were determined. The first consisted of G-21, G-08, G-18, G-17, G-19, and G-07. The second group was formed only by genotypes G-15, G-11, and G-03. The third group consisted of all other genotypes. In the 2017 A evaluation, all genotypes were classified in practically the same group, with the G-12 genotype not being grouped (figure 2, page 8). In the evaluation of 2018, the genotype not grouped with the other genotypes was G-14. In the 2017 B evaluation, genotypes G-07, G-01, and G-04 were classified together in a group, while the other genotypes formed the second group. The joint analysis subdivided the genotypes into a smaller group composed of G-04, G-03, G-02, G-12, G-07 and G-01. The second group included all other genotypes.

To evaluate the contribution of the characteristics to diversity, the technique of main components was used. In all evaluations, the first four main components explained more than 70% of the total variation observed, revealing a reduction in the multidimensional space from nova to just four main orthogonal components (table 4, page 8). Using the weights in the first four components, the characteristics with the greatest participation in the divergence considering all four evaluations were productivity, number of pods per plant, and the number of days for maturation. In the joint analysis, the most prominent characteristics were plant height and productivity. Also worth mentioning is the characteristic of the number of grains per pod in the two evaluations and in the joint analysis. Considering that for the aforementioned characteristics, there was a predominance of cross-interaction (table 3, page 6), it appears that the G × E interaction has a relevant weight in the ordering of distances and, consequently, changes in the groups formed.

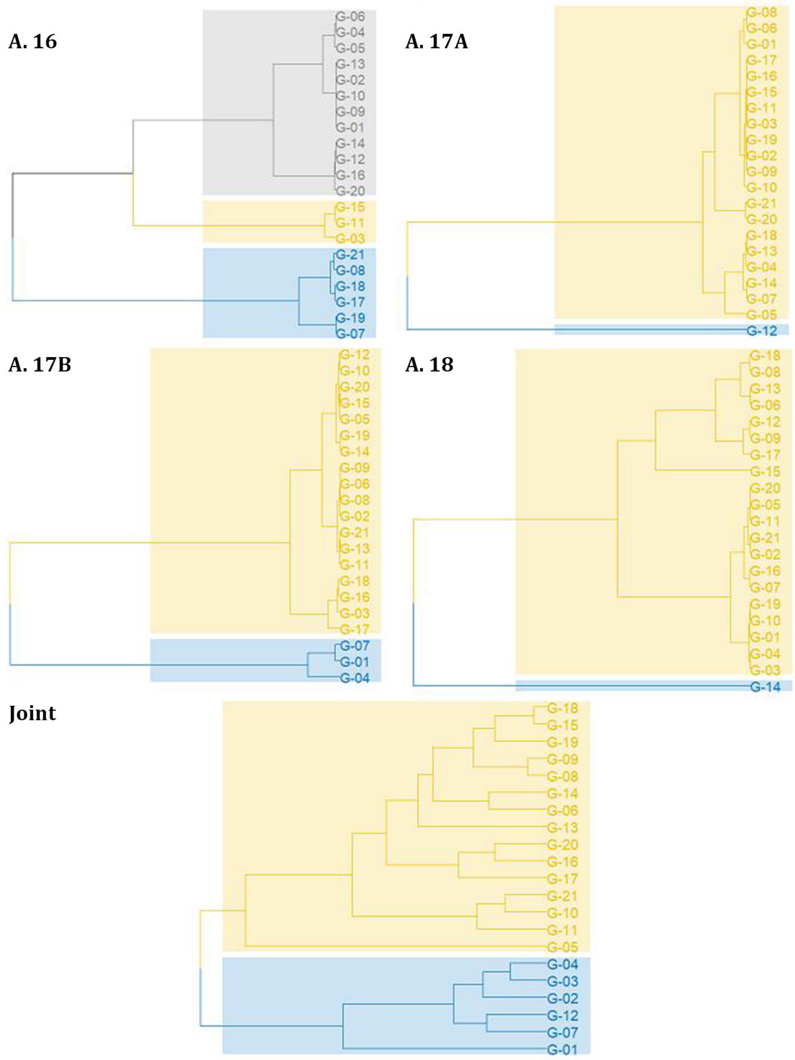

Figure 2. UPGMA dendrograms obtained from the Mahalanobis distance matrix between soybean genotypes evaluated in four tests conducted under semiarid conditions.

Figura 2. Dendrogramas de UPGMA obtenidos de la matriz de distancias de Mahalanobis entre genotipos de soja evaluados en cuatro pruebas realizadas en condiciones semiáridas.

Table 4. Character contribution to the eigenvalue λ_i in the analysis of major components involving soybean genotypes evaluated in four tests conducted under semiarid conditions.
Tabla 4. Contribución del carácter al valor propio λ_i en el análisis de componentes principales que involucran genotipos de soja evaluados en cuatro ensayos realizados en condiciones semiáridas.

Eigenvalue (λ_i)	**Character contribution to eigenvalue λ_i**				
	A.16	A.17A	A.17B	A.18	Conjunct
1	PR (28.02)	NPP (28.82)	NDM (28.85)	NDF (23.18)	PH (29.47)
2	NDM (22.79)	NDM (18.31)	NPP (23.87)	WHS (19.15)	PR (22.09)
3	NPP (18.87)	PR (15.27)	PR (12.73)	PR (17.62)	WHS (14.80)
4	OC (9.87)	PH (10.31)	NVP (11.44)	NVP (13.74)	NVP (11.30)
Total	78.91	72.71	76.89	73.70	77.65

PH: plant height; OC: oil content, in %; NPP: number of pods per plant; NGP: number of grains per pod; WHS: weight of one hundred seeds; PR: productivity, in kg ha^{-1}; NDF: number of days to flower; and NDM: number of days to mature.

AP: altura de la planta; CA: contenido de aceite, en %; NVP: número de vainas por planta; NGV: número de granos por vaina; PC: peso de cien semillas; PR: productividad, en kg ha^{-1}; NDF: número de días para florecer; y NMT: número de días para madurar.

Another result that reinforces the fact that the order of the distances was altered in the different evaluations is that of the estimates of the correlations between the dissimilarity matrices. The correlations were practically non-significant, except for the correlation between the joint analysis and the 2017 B assessment (figure 3).

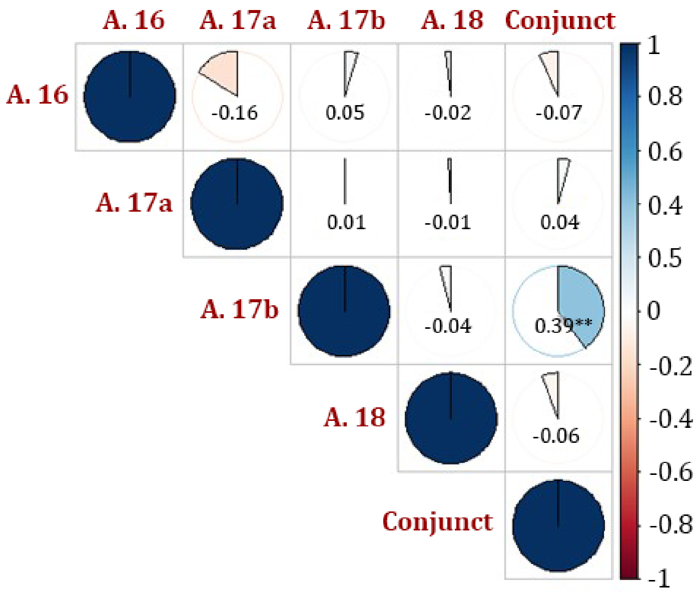

Figure 3. Correlations between Mahalanobis distance matrices of soybean genotypes evaluated in four trials conducted under semiarid conditions.

Figura 3. Correlaciones entre matrices de distancia de Mahalanobis de genotipos de soja evaluadas en cuatro ensayos realizados en condiciones semiáridas.

Although there is vast literature that addresses the effects of the G × E interaction on the selection and identification of cultivars, resulting from the differential behavior of genotypes in the environment, little attention has been paid to the effect of the interaction in diversity studies. Considering that divergence studies are used for determining the initial direction of crossings in order to obtain populations with great variability and a high mean for the characteristics of interest, it is relevant to investigate the effect of the interaction in the formation of heterotic groups, as in the nuclear collections. In the present study, there were differences in the grouping during the four evaluations.

The presence of the genotype–environment interaction effect confused with the genotypic effect in each of the four evaluations, not only resulted in differences in the groups, but also made it difficult to differentiate the evaluated genotypes. The use of the estimates of the free genotypic means of the interaction obtained in the joint analysis may be a more adequate alternative to define the heterotic groups and, consequently, the crossings to be performed. In the present study, crosses were made between the genotypes of the group consisting of G-04, G-03, G-02, G-12, G-07, and G-01 and the genotypes of the second group, in particular, the G-05 genotype. The greatest dissimilarity was found between genotypes G-01 and G-05, shown in red on the heat map (figure 4, page 10). Crosses can be made between the G-01 genotype and the G-13, G-15, and G-18 genotypes.

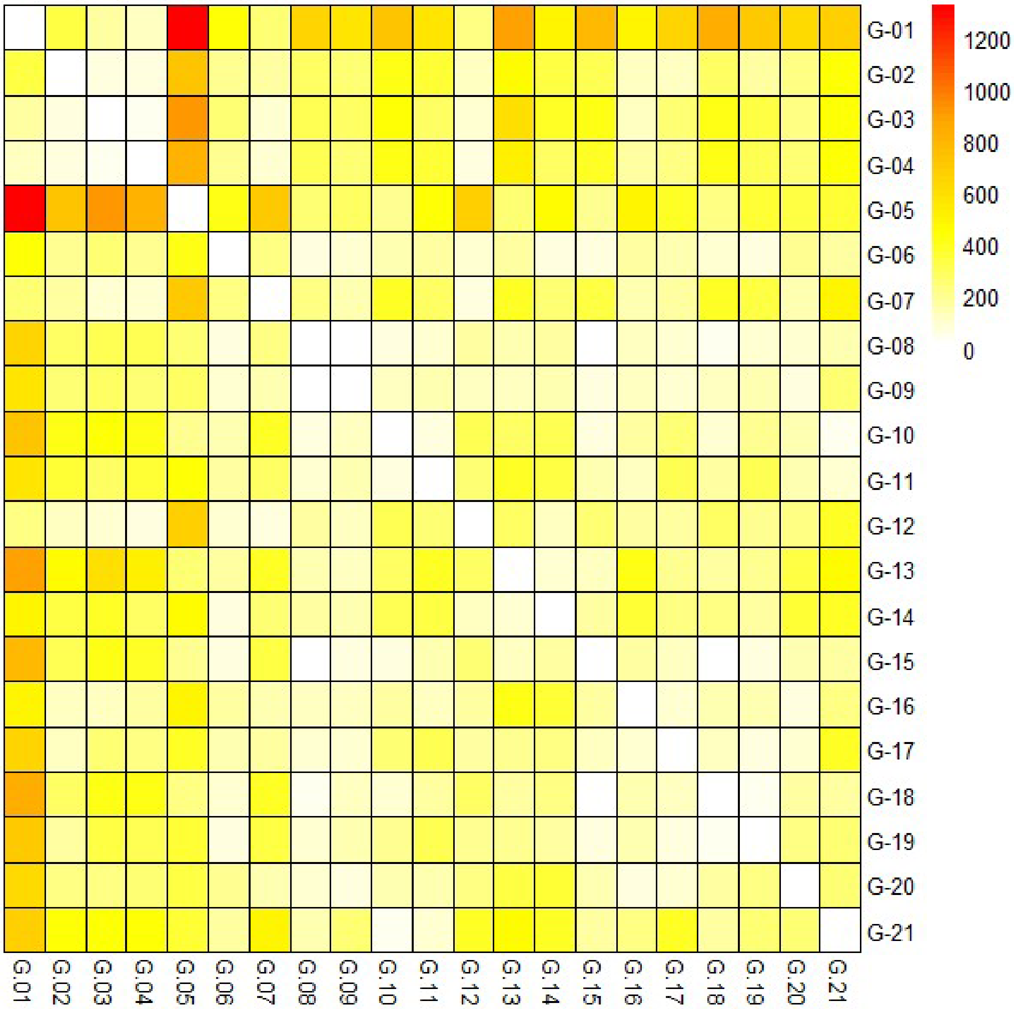

Figure 4. Mahalanobis distances of evaluated soybean genotypes obtained in the joint analysis of four tests conducted under semiarid conditions.

Figura 4. Distancias de Mahalanobis de genotipos de soja evaluados obtenidos en el análisis conjunto de cuatro pruebas realizadas en condiciones semiáridas.

Conclusions

The grouping of soybean accessions is dependent on the evaluation conditions, mainly because of the effects of the genotype por environment interaction. Without the effect of the interaction, the joint analysis allowed us to obtain two groups of genotypes. The most suitable crosses were those carried out between the genotypes BRS Tracajá, BRS Pérola, BRS Carnaúba, M 8644 IPRO, BRS 8590, and BMX OPUS IPRO, and the BRS Sambaíba genotype, especially the one between BMX OPUS IPRO and BRS Sambaíba genotype, which is the most dissimilar.

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Effects of formative and production pruning on fig growth, phenology, and production

Crecimiento, fenología y producción de higuera sometida a intervenciones de formación y poda productiva

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Abstract

Tree pruning influences the phenology of fruit species. The present study aimed to evaluate the growth and phenology of the fig cultivar 'Roxo de Valinhos' subjected to formative and production pruning in the semi-arid region of Piauí. A phenological analysis was performed after formative pruning in 27 fig plants based on the following periods: from formative pruning to the beginning of sprouting, at the beginning of harvest and at its end, and during harvest. Additionally, the variables of branch length, branch diameter, number of leaves, nodes, shoots, inflorescences, and secondary branches were also evaluated. The production pruning treatments (10, 20, and 30 cm) had nine replications each. The Generalized Linear Mixed Model used assumed as fixed factors the branch sizes at pruning (10, 20, and 30 cm), time after pruning (30, 60, 90, and 120 days), and the interaction between factors. The results revealed that figs were well adapted to the semi-arid region of Piauí and showed precocity at all phenological stages compared to those grown in temperate regions. With regard to production pruning, branch size as a function of time did not influence fig development.

Keywords

Ficus carica L. • formative pruning • production pruning • phenological stages • GLMM

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Resumen

La fenología de las especies frutales está influenciada por la poda. En esta perspectiva, el presente estudio tuvo como objetivo evaluar el crecimiento y fenología de la higuera 'Roxo de Valinhos' sometida a intervenciones de formación y poda productiva en la región semiárida de Piauí. Luego de la poda formativa en 27 plantas de higuera, se realizó la evaluación fenológica en base a los períodos comprendidos entre: la poda y el inicio de la brotación; comienzo y final de la cosecha; y duración del período de cosecha. Adicionalmente, se evaluó la longitud, diámetro, número de hojas, número de nudos, número de brotes y número de inflorescencias. Para la poda de producción, las ramas se sometieron a tres tratamientos (10, 20 y 30 cm) con nueve réplicas cada una, evaluando las variables mencionados anteriormente. Para el análisis estadístico se utilizó el GLMM asumiendo como factores fijos el tamaño de la rama al momento de la poda (10, 20 y 30 cm); tiempo después de la poda (30, 60, 90 y 120 días) y la interacción entre factores. El cultivo de la higuera se adaptó a la Mesorregión de Gurgueia Medio Superior y mostró precocidad en todas las etapas fenológicas en relación con las plantas de higuera cultivadas en regiones templadas. En relación con la poda de producción en el cultivo de la higuera, el tamaño de la rama en función del tiempo no influye en el desarrollo del cultivo.

Palabras clave

Ficus carica L. • poda formativa • poda de producción • etapas fenológicas • GLMM

Introduction

Fruit phenology studies are abundant in the scientific literature (22). However, although tree pruning is a widespread practice performed by small and large producers to optimize plant growth, experiments on the ideal branch size for maximum plant development are still incipient, especially in fruit species, and the divergence of most authors (4, 5, 17) with regard to this parameter raises concerns about its actual influence on plant development.

The introduction of temperate species has contributed to diversifying fruit production. Figs stand out in this regard due to their edaphoclimatic adaptability, becoming a viable production alternative as verified by previous studies conducted in northeastern Brazil, especially in the state of Ceará, generating high yields under the climatic conditions of the Apodi Plateau region (9), highlighting the importance of studies on the growth and phenology of temperate crops and their adaptability to warmer conditions (19). From this perspective, pruning also plays an essential role in favoring fig development by improving the canopy structure, controlling fructification, maintaining vigor, and increasing production in new branches.

Pruning management in fruit species is essential for crop development as it may stimulate production and accelerate or anticipate harvest depending on the climatic conditions of the cultivation area (3, 6, 16). However, studies on the ideal pruning intensity of fig branches are still incipient, and this parameter is hypothesized to interfere with fig development and production.

Growth curves are used to analyze phenological data, and since variations may occur among the individuals of a population, it is possible to use a Generalized Linear Mixed Model (GLMM), allowing to include variance components due to non-observed effects, including random effects in the linear predictor, in addition to fixed effects. Introducing random effects in the linear predictor allows modeling the correlation structure of observations performed on the same individual (11). The GLMM aims to describe changes in the mean response of each individual and their relationships with the covariables.

From this perspective, this study aimed to evaluate the growth and phenology of the fig cultivar 'Roxo de Valinhos' subjected to formative and production pruning.

Material and methods

Description of the study area

The study was performed from April 2018 to February 2019 in an orchard belonging to the Fruit Growing Study Group (FRUTAGRO) of the Federal University of Piauí (UFPI), Campus Professora Cinobelina Elvas (CPCE), in the municipality of Bom Jesus, Piauí, Brazil, 9°4'55" S and 44°19'39'' W, at an elevation of 228 m above sea level. The experimental area, located in the semi-arid region of the state of Piauí, has a hot and humid climate with summer-autumn rainfall, with a rainy period from December to May and a dry period from June to November. The climate is classified as Awa according to the Köppen classification, with a mean annual temperature of 26.2°C and mean annual rainfall ranging from 900 to 1,200 mm year^{-1} (15). Figure 1 shows the meteorological data during the experimental period.

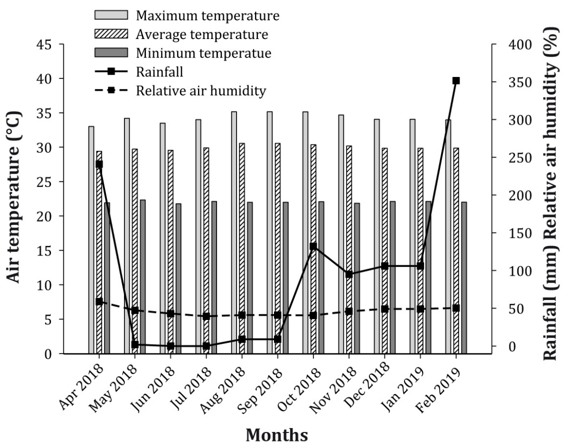

Figure 1. Air temperature (°C), rainfall (mm), and relative air humidity (%) during the experimental period (13).

Figura 1. Temperatura del aire (°C), precipitación (mm) y humedad relativa del aire (%) durante el experimento (13).

Conduction of the experiment

The area has a Dystrophic Yellow Latosol (Oxisol), corresponding to well-drained, slightly undulating, less fertile deep soils whose characteristics are shown in table 1 (soil analysis of the experimental area), determined according to Teixeira *et al*. (2017).

Organic matter - Walkley-Black method; Soil Analysis Laboratory, Campus Professora Cinobelina Elvas - CPCE, Bom Jesus, Piauí.

Materia orgánica - Método Walkley-Black; Laboratorio de análisis de suelos, Campus Professora Cinobelina Elvas - CPCE, Bom Jesus, Piauí.

Table 1. Physical and chemical characteristics of the soil in the 0-20 cm layer.

Tabla 1. Características físicas y químicas del suelo a una profundidad de 0-20 cm.

pH	Organic matter	P	K^+	Ca^{2+}	Mg^{2+}	$H+Al^{3+}$
H_2O	g kg^{-1}	mg dm^{-3}	-----------------cmolc.dm^{-3}-----------------			
6.40	12.50	17.58	0.38	3.00	0.54	3.96
Cu	Fe	Mn	Zn	Clay	Silt	Sand
-----------------------mg dm^{-3}---------------------				--------------g kg^{-1}--------------		
1.12	58.90	18.19	1.46	266.00	31.00	703.00

Plant growth conditions

The fig plants were spaced at 2 m x 1.5 m. A drip irrigation system was adopted, with daily watering for two hours and one emitter per plant (flow rate of 40 L/h). Weed control was performed every 30 days using a hoe and a backpack brush cutter. Fertilization was split into three applications after soil analysis using ammonium sulfate (90 g per plant), single superphosphate (100 g per plant), and potassium chloride (120 g per plant).

In April 2018, formative pruning was performed in 27 fig plants at 50 cm from the ground to allow a single stem per plant. After emergence, the three shoots with the best conditions for canopy formation (vigor and healthiness) were chosen, while others were removed. Finally, one branch per plant was selected for phenological evaluation in the 27 plants of the fig cultivar 'Roxo de Valinhos.'

Phenological analysis was performed over 12 evaluations considering the following periods: from pruning to the beginning of sprouting, at the beginning of harvest and at its end, and during the harvest period. The beginning of sprouting was defined by the emergence of the first shoots; the beginning of harvest was determined by the onset of fruit ripening, with fruit color changing from green to purple; finally, the harvest period ended when most harvested fruits were abnormally colored (uneven and low-intensity purple color) and showed a fibrous pulp, considered unfit for fresh consumption (14).

The following variables were evaluated weekly starting from 30 days after formative pruning: branch length (cm), measured from the base of the branch to its apex with a millimeter ruler; branch diameter (cm), measured at the base of the branch with a portable digital caliper (150 mm, accurate to 0.01 mm); number of leaves per branch; number of nodes per branch; number of shoots per branch; and number of inflorescences per branch. In addition, the number of fruits on each branch was counted every seven days (evaluation days), from their formation until reaching 100% maturation. Harvest was halted when green and small fruits that would not resume ripening were observed.

Production pruning was performed in the 27 fig plants in September 2018. The process consisted of three pruning treatments (10, 20, and 30 cm) with nine replications each. The analyses performed during the present experimental stage were similar to those of the previous stage, but their frequency was fortnightly, not weekly, totaling eight evaluations.

Statistical analysis

The quantitative variables were evaluated using a generalized linear mixed model (GLMM). The model assumed that the response variables could be affected by the following fixed factors: branch size after pruning (10, 20, and 30 cm), time after pruning (30, 60, 90, and 120 days), and the interaction between these two factors (branch size X time after pruning). The effect on each plant was considered a random factor to model the correlation structure between observations of the same individual.

The generalized linear mixed model with a Poisson distribution was specified as follows:

$$Y_{ij}|b_i \sim Poisson(\mu_{ij}), \text{ with } \mu_{ij} > 0$$

$$b_i \sim N(0, \sigma_b^2)$$

$$E(Y_{ij}|b_i) = e^{\eta_{ij}} = Var(b_i) = \mu_{ij}$$

The relationship between the expectation and the linear predictor was given by the *log* link function

$$\eta_{ijk} = log(\mu_{ijk}) = \beta_0 + \beta_1 * B_{1i} + \beta_2 * B_{2i} + \beta_3 * B_{3i} + \beta_4 * T_{1i} + \beta_5 * T_{2i} + \beta_5 * T_{3i} + \beta_6 * T_{4i} + b_i$$

The generalized linear mixed model with a normal distribution was specified as follows:

$$Y_{ij}|b_i \sim Normal(\mu_{ij}), \text{ with } \mu_{ij} = 0$$

$$b_i \sim N(0, \sigma^2)$$

$$E(Y_{ij}|b_i) = 0 \text{ and } Var(b_i) = 1$$

In this case, the relationship between expectation and linear predictor was given by an identity link function unlike the one previously described, in which Y_{ij} is the response variable (branch length, branch diameter, number of leaves, nodes, flowers, fruits, and shoots) of the *i*-th plant with branch size *j* in moment *k* , with *i* = 1, ⍰, 27, *j* = 10, 20, 30, and

$k = 30, 60, 90, 120$; μ_{ij} is the mean number expected in plant *i* with branch size j and in moment *k* ; b_i is the random intercept, and σb is the estimate of variability of the response variable in plant *i*. It should be noted that branch length and diameter (measurable characteristics - continuous data) were evaluated by the normal model, while the other characteristics (counting characteristics - discrete data) followed the Poisson model.

All statistical procedures mentioned below are contained within the Statistical Analysis Systems software (*University Edition* version). Descriptive statistics of the data on the response variables were obtained by the MEANS procedure. These data were analyzed by considering a model of analysis of variance using the Normal and Poisson distributions with the GLIMMIX procedure, and the means were adjusted using the *lsmeans* command (*least-squares means*). Statistical significance was checked by the F-test, and the means were compared by the Tukey-Kramer test. In all analyses, significance was established at $p \leq 0.05$. Spearman's rank correlation coefficient was calculated using the CORR procedure. The SigmaPlot 11.0 software was used to generate the plots of the characteristics evaluated.

Results and discussion

Table 2. Phenology of the fig cultivar 'Roxo de Valinhos' subjected to formative pruning.
Tabla 2. Fenología del cultivar de higo 'Roxo de Valinhos' sometido a poda formativa.

Phenological Stages	Date (MM/DD/YY)	Number of Days	Days after pruning	Mean temperature (°C)	Maximum temperature (°C)
Formative pruning	04/07/2018	01	00	29.4	33.0
Beginning of sprouting	04/12/2018	04	05	29.4	33.0
Beginning of flowering	05/19/2018	27	32	29.71	34.19
Formation of the 1st fruits	06/25/2018	37	42	29.54	33.49
Beginning of harvest	08/13/2018	50	92	30.55	35.15
End of harvest	08/30/2018	17	109	30.55	35.15
Total days of the cycle (pruning/fruit harvest)		109			

In general, the earlier the beginning of sprouting, the shorter it takes for flowering to occur (12). Although a typically temperate fruit species, figs have minimal low-temperature requirements and sprout almost immediately after leaf fall if the temperature remains high (8), as verified at the time of sprouting.

The phenology of fruit species is affected by temperature, and phenological stages such as sprouting, vegetative growth, and harvest can be anticipated at higher temperatures. Under these conditions, plants can absorb and transport nutrients more effectively, favoring their development and vigor.

When studying the fig cultivar 'Roxo de Valinhos' subjected to pruning in the region of Lavras, MG, at 28°C and relative humidity of 67%, Norberto *et al*. (2001) observed 162 days between pruning and harvest for plants pruned in July, whereas, in the present study, 109 days were observed for plants pruned in April. This result suggests that, under the semi-arid conditions of the state of Piauí, the high temperatures shortened the production cycle of the fig cultivar 'Roxo de Valinhos'.

The values found in the present study for the time between pruning and harvest are close to those found by Silva *et al*. (2017) when studying fig phenology and production at 27°C, relative humidity of 68.9%, and July pruning, obtaining 130 days for plants grown in the western region of Rio Grande do Norte.

The crop cycle variations observed may have been due to differences in temperature oscillation, as observed by Souza *et al*. (2009) when studying basal temperatures and the thermal sum of fig plants pruned at different times, resulting in 139 days from pruning to the beginning of harvest for plants pruned in August and grown at 36°C in Botucatu, São Paulo. These results corroborate the present findings as the fig cycle was shortened with the increase of temperature (figure 1, page 15).

Fig fructification requires full sunlight as vegetative buds are quickly differentiated into reproductive buds when temperature increases, increasing the demand for leaf carbohydrates produced by photosynthesis. From this perspective, the high rainfall in April, coinciding with pruning, stimulated the storage of water and nutrients, essential components for bud differentiation.

Plant phenology is dependent on water availability, as clearly observed in semi-arid regions, which alternate between dry and wet periods (19). In Brazil, especially in its northeastern semi-arid region, the conditions and phenological patterns of plants tend to follow the seasonal rainfall fluctuation, causing mild and short-term stresses that result in anticipated flowering and fructification, thus modifying the plant cycle (1).

Branch length, branch diameter, the number of leaves, nodes, and shoots showed a linear growth trend. Their values increased gradually as a function of the evaluation times, reaching the mean values of 31.26 cm per branch, 0.97 cm per branch, 17.56 leaves per branch, 14.24 nodes per branch, and 11.05 shoots per branch after 77 days, respectively (figure 2, page 19).

Branch length showed a nonlinear growth trend (figure 2A, page 19). This variable increased by 0.64 cm per day, reaching the final value of 1.15 cm per branch after 77 days. According to figure 2B (page 19), branch diameter reached its maximum growth rate after 63 days, corresponding to 32.61 mm. Considering the initial branch diameter, this variable increased by 17.88%.

High vegetative growth rates occur due to high temperatures after pruning (figure 1, page 15), resulting in competition between processes that demand photoassimilates, such as branch and shoot growth and fruit development.

The number of leaves also showed a nonlinear growth trend as evaluations succeeded, reaching 33.70 leaves per branch after 77 days (figure 2C, page 19). These values are higher than those reported by Silva *et al*. (2011) when studying fig growth under different conditions, obtaining the lowest and highest numbers of leaves per branch of 13.32 and 15.87, respectively.

Celedonio *et al*. (2013) analyzed three types of fig cultivation in Limoeiro do Norte, CE, and observed that plants grown in the field had 44 leaves per branch after 90 days. However, it should be noted that the climatic conditions of Limoeiro do Norte differ from those of the present study.

The number of leaves decreased in the period from 42 to 49 days after pruning (figure 2C, page 19), consequently affecting the number of nodes (figure 2D, page 19) and shoots (figure 2E, page 19) as these structures emerge from the leaf axil. This reduction may have been due to the high temperatures and low relative humidity of the region (figure 1, page 15), reducing plant metabolic activity for ± 2 months, as evidenced by leaf fall and the presence of fewer nodes and shoots.

With regard to the number of fruits per branch, despite the value of 0.67 for this parameter (figure 3, page 20), which is considered below-average compared to studies such as the one by Silva *et al*. (2017), it should be noted that the plants were still in early development. Moreover, the primary purpose of formative pruning is not to produce marketable fruits immediately but rather to develop good canopy architecture and vegetative vigor, stimulating nutrient uptake and storage for the reproductive period and preparing the plants for production pruning.

Vegetative development depends on the uptake and processing of essential materials for plant growth, such as water, energy, carbonic gas, and nutrients. This process implies changes in the internal relationships of the crop with the external environment and is directly associated with the cultivation area, rainfall, temperature, humidity, and severe stresses to which plants may be subjected, such as pruning (2).

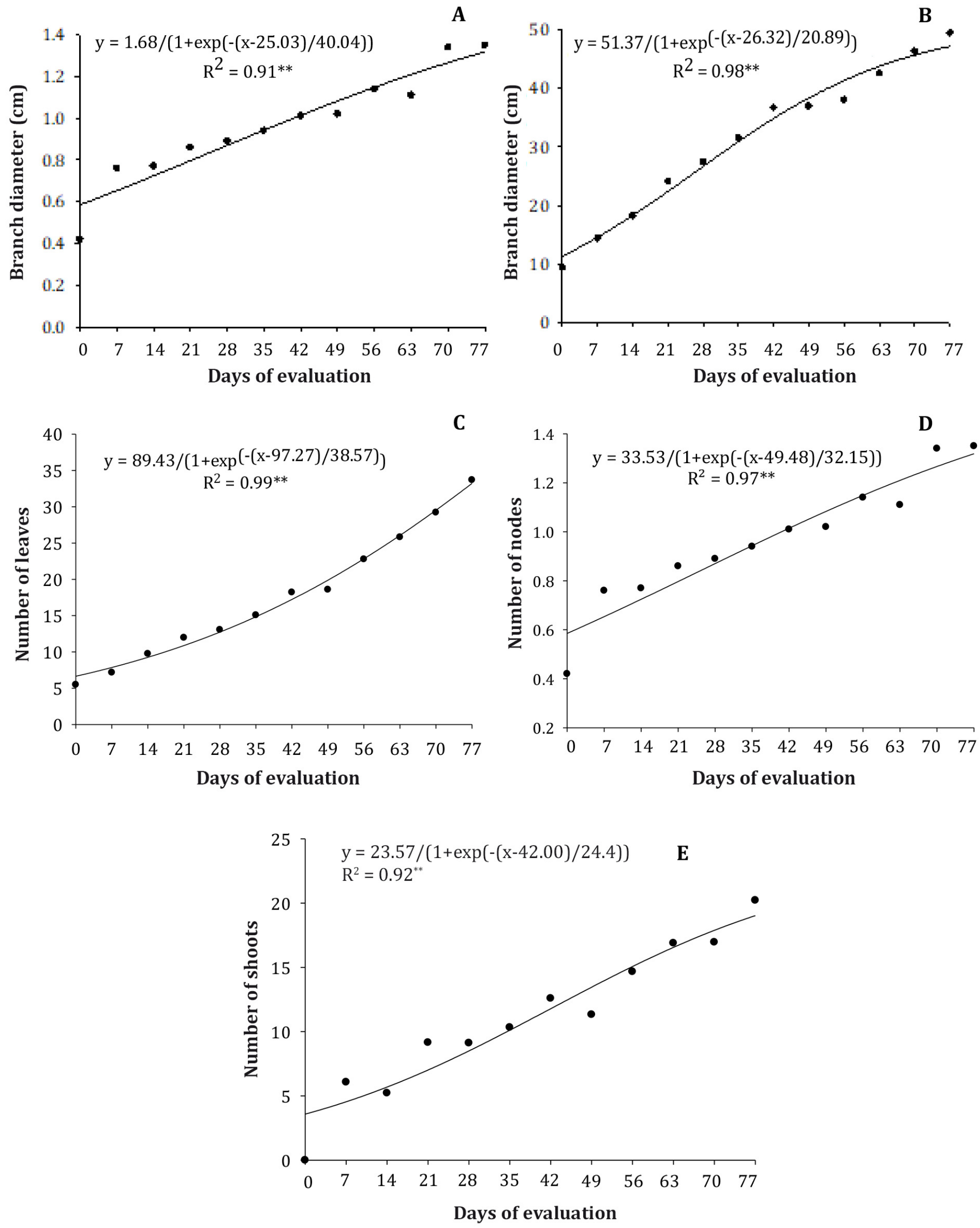

Figure 2. Phenology of the fig cultivar 'Roxo de Valinhos' subjected to formative pruning as a function of the number of days after pruning. Branch length (3A); Branch diameter (3B); Number of leaves (3C); Number of nodes (3D); Number of shoots (3E).

Figura 2. Fenología del cultivar de higo 'Roxo de Valinhos' sometido a poda formativa en función del número de días después de la poda. Longitud de la rama (3A); Diámetro de la rama (3B); Número de hojas (3C); Número de nodos (3D); Número de brotes (3E).

The fig plants showed uniformity and typical branch growth after production pruning, although with late sprouting and fruit formation compared to the period after formative pruning, with an average of 70 days. There was a 49-day period from fruit formation to the beginning of harvest, one day shorter to formative pruning, after which the fruits were fit for fresh consumption (table 3, page 20).

The high temperatures explain the late sprouting and fruit development observed, inducing physiological delay as the plants responded with slower growth regardless of branch size.

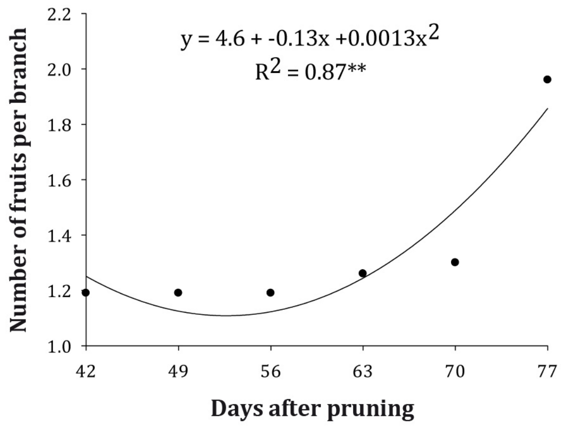

Figure 3. Number of fruits per branch in fig plants as a function of the number of days after pruning.

Figura 3. Número de frutos por rama en higueras en función del número de días después de la poda.

Table 3. Phenology of the fig cultivar 'Roxo de Valinhos' subjected to production pruning.

Tabla 3. Fenología del cultivar de higo 'Roxo de Valinhos' sometido a poda de producción.

Phenological Stages	Date (MM/DD/YY)	N° Days	Days after pruning	Mean temperature (°C)	Maximum temperature (°C)
Formative pruning	09/28/2018	01	00	30.55	35.15
Beginning of sprouting	10/02/2018	04	04	30.55	35.14
Beginning of flowering	12/18/2018	77	81	29.84	34.04
Formation of the 1st fruits	12/22/2018	04	85	29.84	34.04
Beginning of harvest	02/09/2019	49	134	33.96	33.96
End of harvest	02/28/2019	19	153	33.96	33.96
Total days of the cycle (pruning/fruit harvest)		153			

Phenological studies conducted by Ferraz *et al*. (2017) with the fig cultivar 'Roxo de Valinhos' pruned at 20.3°C and relative humidity of 58.3% in the region of Botucatu, SP, showed that the period between pruning and sprouting was 13.69 days for plants pruned in August, while the period between the beginning of sprouting and the beginning of harvest was 155 days. These values are above those of the present study and highlight that the production cycle of the fig cultivar 'Roxo de Valinhos' was shortened due to the high temperatures of the semi-arid region of Piauí.

The ratio between the generalized chi-square statistics and its degrees of freedom is close to 1 (table 4, page 21). This indicates that data variability was adequately modeled and there was no residual overdispersion (variance higher than the mean).

No significant difference was observed ($p>0.05$) for branch size and the branch size x day interaction with regard to the characteristics evaluated. However, there was a significant difference for the *day* factor (table 5, page 21). The variance component model was adopted to structure the residual (co)variance of measurement variations for each individual.

Table 4. Analysis of variance of the effects included in the model for the studied characteristics in fig plants.

Tabla 4. Análisis de varianza de los efectos incluidos en el modelo para las características estudiadas en plantas de higuera.

Characteristic	χ^2/DF	Effect	numerator DF	F	*p-value*
Branch Length	1.17	Branch size	2	0.49	0.6117
		Day	3	32.23	<.0001
		Branch size*day	6	0.17	0.9835
Branch Diameter	0.60	Branch size	2	0.23	0.7942
		Day	3	43.34	<.0001
		Branch size*day	6	0.08	0.9983
Number of Leaves	1.04	Branch size	2	0.22	0.8022
		Day	3	39.6	<.0001
		Branch size*day	6	0.3	0.9354
Number of Nodes	1.20	Branch size	2	0.14	0.8678
		Day	3	69.36	<.0001
		Branch size*day	6	0.04	0.9998
Number of Flowers	1.00	Branch size	2	1.11	0.3353
		Day	3	20.4	<.0001
		Branch size*day	6	0.82	0.5537
Number of Fruits	0.98	Branch size	2	0.37	0.6937
		Day	3	18.57	<.0001
		Branch size*day	6	0.37	0.8981
Number of Shoots	1.19	Branch size	2	0.03	0.9669
		Day	3	74.09	<.0001
		Branch size*day	6	0.10	0.9962

χ^2 - chi-square;
DF - degree of freedom.
χ^2 - chi-cuadrado;
DF - grado de libertad.

Table 5. Adjusted means of fig characteristics as a function of the number of days after pruning.

Tabla 5. Medias ajustadas de las características del higo en función del número de días después de la poda.

Day	Branch Length	Branch Diameter	Number of Leaves	Number of Nodes	Number of Flowers	Number of Fruits	Number of Shoots
30	7.6019 d	0.5870 d	7.8800 c	5.7771 c	0.0000 b	0.0000 b	1.3302 c
60	23.4663 c	0.7804 c	15.0583 b	11.9244 b	0.0000 b	0.0000 b	10.3686 b
90	35.4181 b	1.1104 b	17.2563 a	17.4073 a	0.0374 b	0.0000 a	15.6216 a
120	41.1842 a	1.2389 a	18.5393 a	19.6576 a	0.7407 a	1.1481 a	14.9193 a

Means followed by different lowercase letters, per characteristic, differ from each other by the Tukey-Kramer test ($p<0.05$).
Medias seguidas de diferentes letras minúsculas, por característica, se diferencian entre sí por la prueba de Tukey-Kramer (p <0,05).

The Tukey-Kramer test highlighted that the number of days after pruning influenced plant development. This was expected as the longer the time, the more the crop develops. However, there was no significant difference in the number of leaves, nodes, and shoots from 90 to 120 days after pruning, implying that branch size reached a ‘standard value’ 90 days after pruning. In other words, the plants reached statistically similar measurements, growing and developing as if they had not been subject to production pruning.

In the present experience, the optimal climatic conditions for plant development should also be noted, with well-distributed rainfall, high temperatures, and high relative air humidity, promoting uniformity among plants even when subjected to the stress caused by production pruning.

Branch length showed a linear growth trend (figure 4A, page 22), with a constant daily increase of 0.34 cm. After 120 days, the mean growth rate was 0.99 cm per branch. On the other hand, figure 4B (page 22), shows that branch diameter reached its maximum growth rate of 32.61 mm after 60 days. Based on the initial branch diameter, this variable increased by 14.66%. Branch length and diameter showed linear growth trends, and their values increased gradually as a function of the evaluation times, reaching 41.18 cm per branch and 1.24 cm per branch, after 120 days. A similar situation occurred for the number of nodes (figure 4D, page 22) (19.66 nodes per branch).

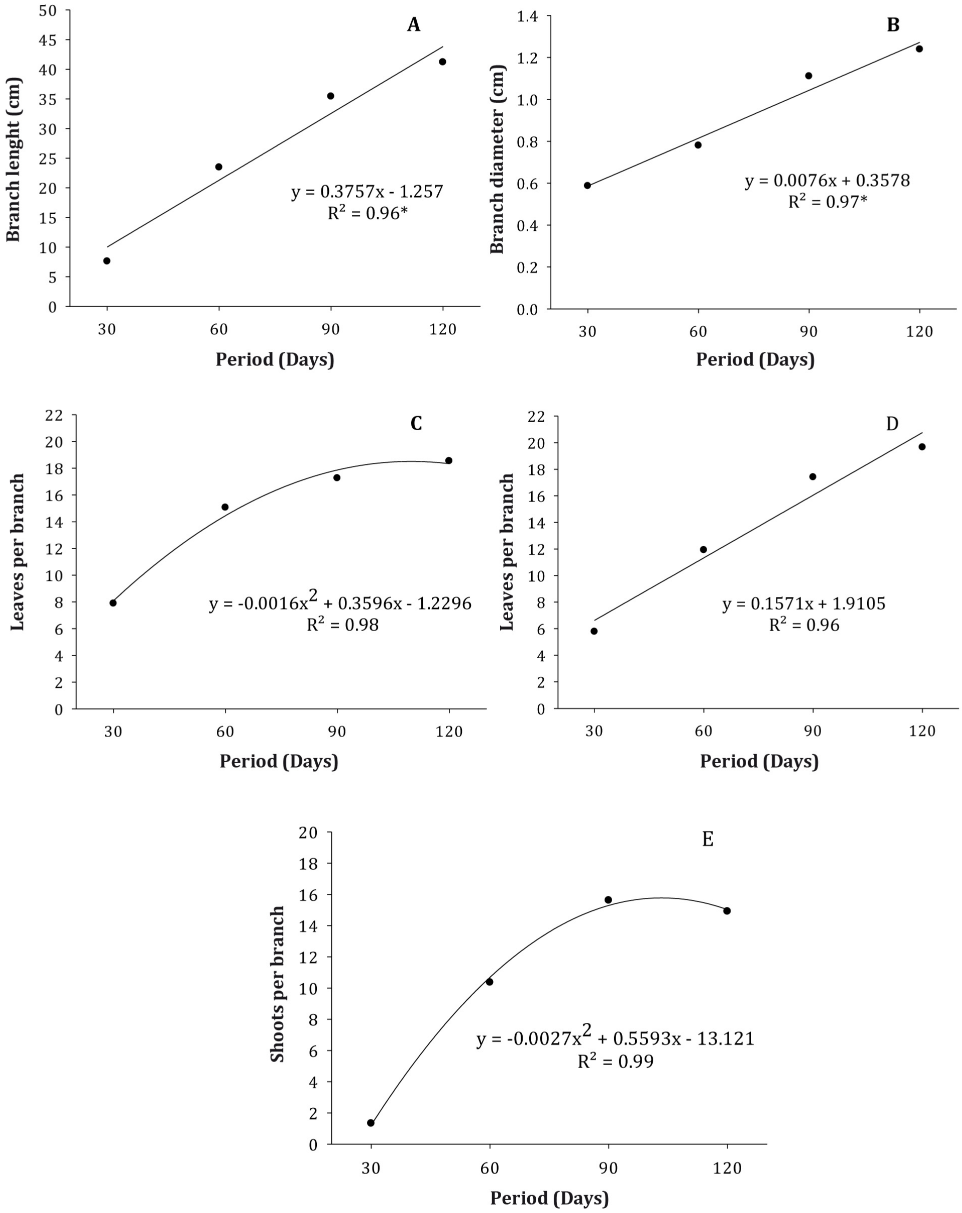

Figure 4. Phenology of the fig cultivar 'Roxo de Valinhos' under repeated measurements over time.

Figura 4. Fenología del cultivar de higo 'Roxo de Valinhos' en función de mediciones repetidas a lo largo del tiempo.

A positive response was verified for the number of leaves (figure 4C) and shoots (figure 4E), which showed quadratic growth curves. The measurements reached their maximum potential 90 days after production pruning, with 18.54 leaves per branch and 14.91 shoots per branch. These observations show that, in general, the responses of closer times in repeated measures experiments are more strongly correlated than those of more distant times. The low rainfall rates after pruning may have delayed fruit formation and contributed to the non-significance of the number of fruits observed in the present study, especially for the cultivar 'Roxo de Valinhos,' which fructifies well under ideal rainfall conditions. However, the results for the vegetative phase were promising.

Some of the characteristics evaluated were correlated, such as branch length with branch diameter and the number of nodes with the number of shoots (table 6, page 23). Vigorous fig branches develop both in length and diameter, supporting the leaves properly.

Table 6. Spearman's rank correlation coefficient for the studied characteristics in fig plants.

Tabla 6. Coeficiente de correlación de rangos de Spearman para las características estudiadas en plantas de higuera.

	Branch Diameter	Number of Leaves	Number of Nodes	Number of Flowers	Number of Fruits	Number of Shoots
Branch Length	0.91 (p<.0001)	0.87 (p<.0001)	0.91 (p<.0001)	0.38 (p<.0001)	0.40 (p<.0001)	0.86 (p<.0001)
Branch Diameter	-	0.85 (p<.0001)	0.91 (p<.0001)	0.42 (p<.0001)	0.45 (p<.0001)	0.85 (p<.0001)
Number of Leaves	-	-	0.85 (p<.0001)	0.38 (p<.0001)	0.37 (p<.0001)	0.84 (p<.0001)
Number of Nodes	-	-	-	0.40 (p<.0001)	0.42 (p<.0001)	0.92 (p<.0001)
Number of Flowers	-	-	-	-	0.86 (p<.0001)	0.31 (0.0013)
Number of Fruits	-	-	-	-	-	0.28 (0.0038)

The correlation between the number of nodes and the number of shoots was high as nodes are necessary for shoot emergence. However, in cases of very severe pruning, shoots may emerge in random spots of the plant, which did not occur in the present study.

Conclusion

The fig cultivar 'Roxo de Valinhos' was well adapted to the semi-arid region of Piauí and showed precocity at all phenological stages compared to fig plants cultivated in temperate regions.

With regard to production pruning, branch size as a function of time did not influence fig development according to the characteristics evaluated.

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Desiccation, storage and physiological quality of *Phoenix roebelenii* O'Brien (Arecaceae) seeds

Desecación, almacenamiento y calidad fisiológica de las semillas de *Phoenix roebelenii* O'Brien (Arecaceae)

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ABSTRACT

Phoenix roebelenii is a palm species widely used in Brazil due to its exuberant leaves and adaptability to different climatic variations. For proper propagation, detailed information about seed viability, vigor and longevity subjected to dehydration and storage is yet to be obtained. For this purpose, the present research comprised two stages. The first one aimed at evaluating the response of seeds to desiccation and storage based on formal protocols. In the second stage, seeds were desiccated to 33, 30, 25, 23, 20, 18, 16, 12, 10 and 7% of water content by monitoring water loss in a convection oven at 35±2°C. After reaching each established water content, the seeds were subjected to germination tests. At 65 days after sowing, root length, root area, volume and diameter, shoot and eophyll length, and total seedling fresh and dry weights, were measured. Significant increases in germination and eophyll length were observed for lower seed water contents. Aversely, collar diameter decreased with seed desiccation.

Keywords
palm tree • Arecaceae • seed water content • vigor • viability

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Resumen

Phoenix roebelenii es una especie de palma muy utilizada en Brasil debido a sus hojas exuberantes y su adaptabilidad a diferentes tipos de clima. Por esto, resulta importante obtener información detallada sobre la viabilidad, vigor y longevidad de sus semillas deshidratadas y almacenadas. La presente investigación comprendió dos etapas. La primera tuvo como objetivo evaluar la respuesta de las semillas a la desecación y almacenamiento con base en protocolos formales. En la segunda, las semillas se desecaron hasta alcanzar el 33, 30, 25, 23, 20, 18, 16, 12, 10 y 7% de contenido de agua. Luego de alcanzar cada nivel de agua establecido para cada tratamiento, las semillas fueron sometidas a pruebas de germinación. A los 65 días después de la siembra se midió: longitud del sistema radicular y del brote, el área, volumen y diámetro del sistema radicular, la longitud del eófilo y el peso fresco y seco de las plántulas. Por um lado, se observó un aumento significativo en el porcentaje de germinación y en la longitud del eófilo en función del menor contenido de agua de la semilla. Por otro lado, el diámetro del collar disminuyó como consecuencia de la desecación de la semilla.

Palabras clave

palmera • Arecaceae • contenido de agua de la semilla • vigor • viabilidad

Introduction

Palms are among the most cultivated plants worldwide. The species domestication, linked to the development of agriculture, represents a significant step in human development (3, 22).

The species *Phoenix roebelenii* O'Brien is indigenous to the Southeast of Asia. In Brazil, known as dwarf date palm or phoenix palm, is widely used in landscape design projects, parks, gardens, streets and interior design. It is mainly appreciated for its height, exuberant leaves and edaphoclimatic adaptability (9, 20, 21).

Following seed dispersal, germination is one of the most important physiological mechanisms for perpetuation of the species (6), defining the existence of a community in a certain habitat. Nevertheless, for germination success, several specific environmental factors, such as water, light and suitable temperature must range within certain values (7).

Eric Roberts, studying seeds storage, proposed a definition for recalcitrant seeds in the 70s, (1). Seeds showing tolerance to desiccation and storage at negative temperatures were called orthodox, while seeds that lost their viability when subjected to these conditions, were called recalcitrant (1). For orthodox seeds, during the latest stages of maturation, when desiccation occurs, metabolism becomes quiescent, and orthodox seeds gain desiccation tolerance, allowing seed storage without viability loss (4). However, despite the low metabolic activity after seed desiccation, seeds do deteriorate over time, becoming evident and quantifiable, after rehydration (16).

In this sense, desiccation tolerance (TD) can be defined as seed capability to maintain viability after loosing cell water content without showing irreversible damages (14, 22). Additionally, seed critical water content can be understood as the minimal water content ensuring survival, that is, without irreversible damage. This value is approximately, 0.07 g H_2O g dry weight^{-1} for orthodox seeds and 0.2 g H_2O g dry weight^{-1} for recalcitrant seeds (24). Thus, TD is an evolutionary characteristic allowing growth and reproduction even after severe dehydration, sometimes above 90% (12).

Variations in seed responses to storage suggest the existence of complex mechanisms. Their understanding inspire new technologies ensuring seed availability along time (24).

The present research is motivated by the interest in *P. roebelenii* as an ornamental palm and the need to preserve the species variability. Our objective was to analyze seed physiological quality after desiccation and storage.

Material and methods

Study area, seed harvesting and processing

Fruits of *P. roebelenii* were manually harvested from plants grown in private gardens in Campos dos Goytacazes, Rio de Janeiro - Brazil. Fruits were immersed in water for 72 hours. Nextly, all seeds were manually extracted by friction in a steel sieve, removing all residues by rinsing the seeds in running water. Seed surface was disinfested with 2% sodium hypochlorite for 5 minutes. After rinsing, excess water was removed with toilet paper and seeds were left to air dry in the shade, for 24 hours.

Physiological classification after desiccation and storage

The physiological classification of *P. roebelenii* store seeds was based on Hong and Ellis (1996), adjusting the drying temperature in the convection oven to 35±2°C. Firstly, initial seed water content was determined as described in Brasil, Ministério da Agricultura, Pecuária e Abastecimento (2009). Afterwards, each step of the protocol was carried out with eight replications, each with 50 seeds, corresponding to the different seed lots. Desiccation time was estimated using the equation for viability evaluation (11):

$$MR = [((100-Ti)/(100-Td)) \times Mi]$$

where:
MR = sample mass reaching the desired moisture content
Ti = initial water content of the seed lot
Td = desired water content
Mi = initial mass of the seed sample (11).

At 12% water content, immediately after drying, 400 seeds separated in 16 fractions of 25 seeds, were used for seed viability evaluation. Emergence tests were conducted according to the methodology described below. The remaining seed lots, 200 seeds, remained in the desiccation chamber (convection oven) until reaching 5.03% seed water content. Then they were split in two sets, one for a new emergence test, while the other with 5.03% water content, was kept in a glass jar at -20°C, for 90 days. After this storage period, another germination test evaluated their physiological quality.

Desiccation and treatments

After mesocarp extraction, 33.92% seed initial water content was determined as described in Rules for Seed Analysis (5). The other treatments (33; 30; 25; 23; 20; 18; 16; 12; 10 and 7% water content) were obtained by monitoring seed water loss after adjusting oven temperature to 35±2°C, adapting the methodology proposed (11).

In the drying process, groups of 25 seeds were laid in gerbox boxes, all with initial known masses. The drying process was monitord by periodical weighting at one-hour intervals during the first 12 hours; and at six-hour intervals until the final desired seed water contents. For this purpose, the Hong and Ellis equation was used (11). Once the desired water contents were reached, the sample was homogenized, separated in 16 fractions of 25 seeds and sealed in hermetical glass flasks in a refrigerator at 7°C, temporarily, until all treatments were processed, that is, at 72 hours after starting seed desiccation.

Physiological evaluation of seed quality

Firstly, seed water content was determined by weighing (10-4 g precision) 30 replications with 30 seeds each. Then, the seeds were oven-dried at 105 ± 3°C, for 24 hours (5). The results were expressed in average percentage of humidity. Afterwards, the mass of a thousand seeds was determined as in Brasil, Ministério da Agricultura, Pecuária e Abastecimento (2009).

Germination tests were carried out with 16 subsamples, 25 seeds each. Seeds were sown on two layers of germitest paper and covered with a third layer. All paper layers had been previously imbibed with distilled water, 2.5 times their weights. Germination rolls were kept in a gemination chamber (BOD type), with 16/8 hours photoperiod, at 30°C.

Daily counting of normal seedlings (5), was then expressed as germination percentage. Seeds were considered germinated at primary root protrusion ≥ 2.0 mm. Concomitantly, the first germination was registered as the number of seeds germinated at 20 days after sowing. Germination speed index (GSI) was calculated as proposed by Maguire (1962). Number of germinated seeds was daily monitored until day 45 after sowing, after which it remained constant.

Seedling morphological evaluations

At 65 days, the following evaluations were made: i) length of the longest root (cm), from collar to tip of the longest root; ii) root/shoot length (cm), area (cm^2), volume (cm^3) and diameter (mm) were obtained using images and the WinRhizo Pro 2007a software, coupled to a scanner. iii) eophyll length - measured with a digital calliper, between the base and the apex; iv) collar diameter, measured with a digital calliper at seedling base; v) total fresh and dry seedling weights. Dry weight was obtained by conditioning each separate seedlings in Kraft paper bags kept in a convection oven at 65°C, until constant weight. Fresh and dry weights were determined with a 10-3 g precision scale.

Statistical analyses

The experimental design consisted of completely randomized blocks with 10 treatments (water content %) and eight replications of 25 seeds. Data were subjected to analysis of variance and means were compared by Tukey test at 5% probability, prior normality and homoscedasticity verification. Data analyses were done with R Core Team software.

Results and discussion

P. roebelenii seeds had an initial water content of 33.9% (figure 1) and were desiccated for 72 h suffering an abrupt drop of water content in the first 15 hours, reaching, approximately, 10.0%. Later, at 36 hours, a relative stabilization in seed water loss was achieved with about 7.0%, finally dropping to 5.03% at 72 hours (figure 1).

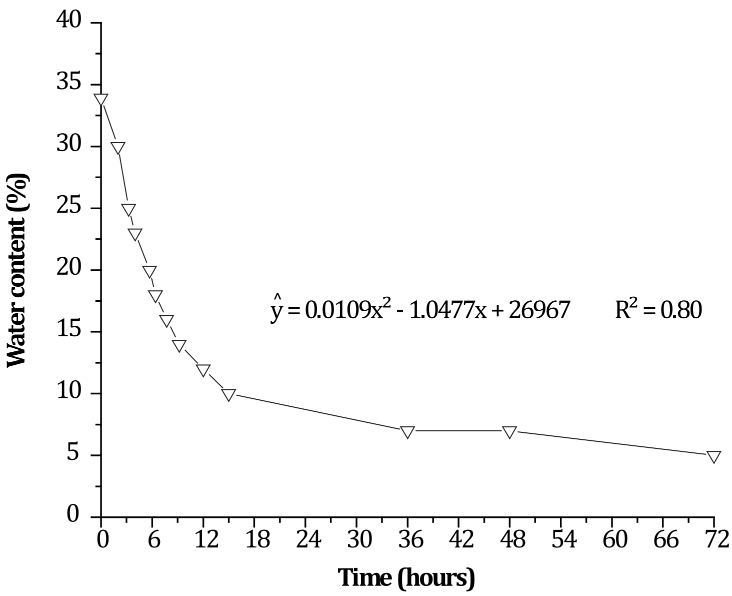


Figure 1. Water content (%) of *Phoenix roebelenii* seeds subjected to desiccation in a convection oven at 35°C. Significant at 5% ($p < 0.05$) probability.

Figura 1. Contenido de agua (%) de semillas de *Phoenix roebelenii* sometidas a desecación en un horno de convección a 35°C. Significativo al 5% ($p < 0,05$) de probabilidad.

Data show that *P. roebelenii* seeds preserved certain germination stability after reduced water content and storage (table 1). At 33.92% water content, germination was 87.5%, reaching the highest percentage (92,5%) at 10.0% water content. Even after desiccation and hermetic storage for 90 days at -20°C, *P. roebelenii* seeds showed 88.0% germination (table 1), indicating high desiccation tolerance and adequate storage at low temperatures. Thus, these seeds can be classified as orthodox (11). However, studying desiccation tolerance (TD) and viability during seed storage of the *S. schizophylla* palm, decreasing seed moisture from 21.3 to 11.6% caused more than 50% drop in germination percentage (3). Furthermore, no germination occurred below 11.6%, allowing *S. schizophylla* seeds to be classified as recalcitrant (3). In brief, different palm species show different behaviors in relation to seed recalcitrance, requiring different methodologies for storage and propagation. Studying seed desiccation sensitivity in *Oenocarpus bacaba* palm, reported that germination is significantly influenced by water content (2). At 41.7% moisture, *O. bacaba* seeds presented 70% germination. However, at 26.7%, they showed 100% mortality. These authors also reported that, in more developed seedlings, plant organogenesis, such as eophyll and cataphyll, is impaired (2). In this context, seed high water content during storage can favor deterioration processes by increasing respiration, followed by membrane degradation, microorganisms proliferation, and reduced germination and vigor (1, 4). On the other hand, investigating desiccation tolerance of *Butia odorata* seeds, Fior *et al.* (2019) concluded an orthodox behavior, reaching a viability index of 90% at 6.14% moisture. Furthermore, Nascimento *et al.* (2010) observed no physiological effects on *Euterpe oleraceae*, seeds, cultivar BRS Pará, for up to 37.4% seed desiccation. However, water contents below 37.4% progressively favored deterioration, achieving null seed viability at 15.1%. The authors reported seed conservation of 270 days after storage at 37.4% water content and 20°C.

Table 1. Mean water content (%) and germination of *Phoenix roebelenii* seeds subjected to desiccation and storage.

Tabla 1. Contenido medio de agua (%) y germinación de semillas de *Phoenix roebelenii* sometidas a desecación y almacenamiento.

	Desiccation				
Water content (%)	33.92	20.0	10.0	5.03	5.03 and hermetically stored for 90 days at -20°C
Germination (%)	87.5	89.0	92.5	90.5	88.0

Desiccation tolerance can be defined as seed capability to maintain viability after losing cell water content without presenting irreversible damages (14, 22). Table 1 indicates that *P. roebelenii* seeds did not suffer significant viability or vigor loss. Nevertheless, ongoing research has modified seed classification as recalcitrant, intermediary, or orthodox. Given the varied responses to desiccation and temperature tolerance, along with viability maintenance, different degrees of recalcitrance have been proposed, considering longevity during storage, dormancy and vigor (1).

Furthermore, many authors have acknowledged the need for new technologies allowing seed classification according to desiccation tolerance. Researchers state that besides TD and longevity, any characteristics that might distinguish different degrees of recalcitrance should be considered for seed classification (1). For instance, habitat, water content at 50% seed viability, dormancy, germination percentage immediately after dispersion, and maturation degree, among others. Previous reports on other *Phoenix* palm species considered *Phoenix dactylifera* seed as orthodox and *Phoenix canariensis*, *Phoenix rupicola*, *Phoenix sylvestris* and *Phoenix reclinata* as probably non-orthodox or intermediate (18, 19).

Table 2 contains the descriptive statistics: mean and extreme values, and coefficient of variation for first germination count (FGC), root length (RL), root superficial area (SARS), root diameter (DR), root volume (VRS), seedling fresh weight (TFW) seedling dry weight (TDW). None of them were significantly affected by the treatments at the significance level of 5% probability. Only the FGC and TWD variables did not meet the normal distribution assumption.

Table 2. Mean and extreme values, and coefficient of variation (CV) for first germination count (FGC), root length (RL), root superficial area (SARS), root diameter (DR), root volume (VR), total seedling fresh weight (TFW) and seedling total dry weight (TDW) of *Phoenix roebelenii*.

Tabla 2. Valores medios y extremos y coeficiente de variación (CV) del primer recuento de germinación (FGC), longitud del sistema radicular (RL), área superficial del sistema radicular (SARS), diámetro del sistema radicular (DR), volumen del sistema de clasificación (VR), peso fresco total de plántulas (TFW) y peso seco total de plántulas (TDW) de *Phoenix roebelenii*.

Variables		Statistical parameters			
	Significant	Low	Average	High	CV (%)
FGC (%)	ns	4.0	13.7	20.0	47.49
RL (cm)	ns	6.61	8.29	9.53	8.99
SAR (cm^2)	ns	13.83	16.67	18.74	9.35
DR (cm)	ns	0.42	0.48	0.53	5.43
VR (cm^3)	ns	0.16	0.19	0.23	10.71
TFW (mg)	ns	245.6	272.08	302.0	3.89
TDW (mg)	ns	84.70	92.23	138.7	9.15

Legend: ns = non-significant. * Significant at 5% ($p<0.05$) probability.

Leyenda: ns = no significativo. * Significativo al 5% ($p<0,05$) de probabilidad.

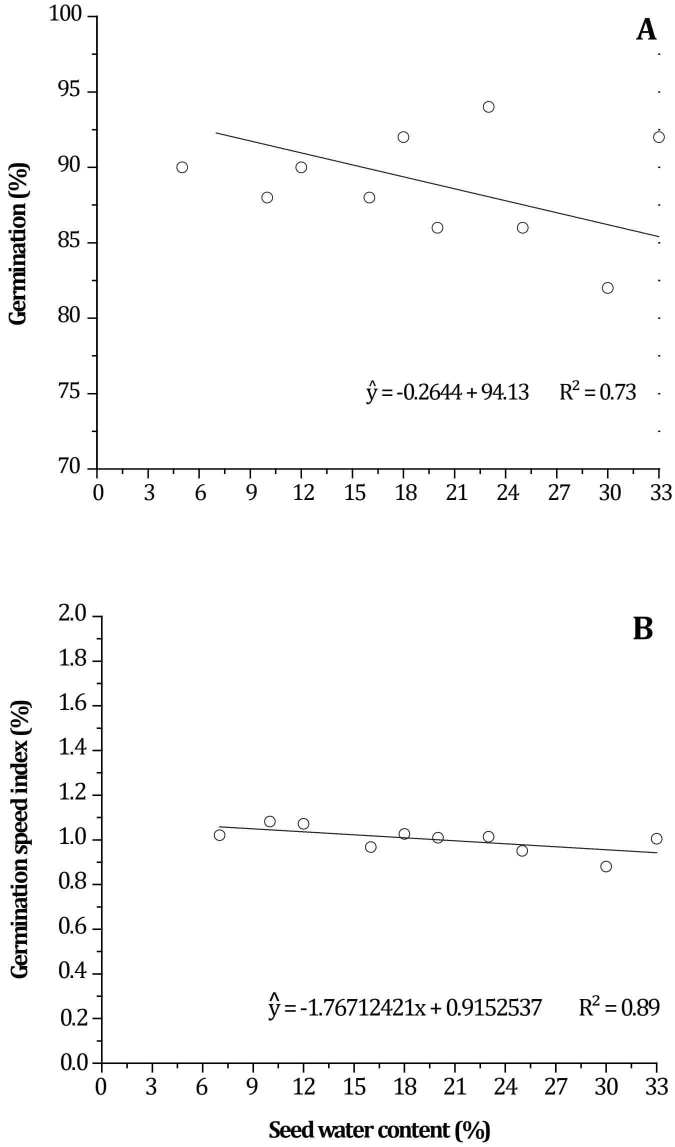
The FGC coefficient of variation (CV) was extremely high (47.49%). This variable, along with TDW did not show normal distribution (table 2). The RL had a low CV (8.99%). SARS, DR and VR showed intermediate values, 1874 cm^2, 0.53 cm and 0.23 cm^3; respectively (table 2). FDW showed normal distribution (table 2).

Germination percentage (PG) and gemination speed index (GSI) significantly increased with decreasing seed water content. When seed water contents ranged between 5 and 12%, the highest percentages of germination were observed (figure 2A, page 31). The highest GSI and IVG (1.14 and 1.15) were observed when seed water contents were 10 and 12%, respectively (figure 2B, page 31). These indices represent key information for establishing normal and vigorous seedlings, indicating seed physiological quality (15).

The results indicate that *P. roebelinii* seeds are orthodox. This considers critical water content as the lowest water content allowing survival or not causing irreversible damage. This value is approximately 0.07 g H_2O g dry weight^{-1} for orthodox seeds and, approximately, 0.2 g H_2O g dry weight^{-1} for recalcitrant seeds (24). In *P. roebelenii* seeds, 98% PG was reported when seed water content was 30% (18), while at 8%, germination dropped to 90%. However, *Caryota urens* L. seeds with an initial moisture content of 34%, presented PG of 95%, whereas total loss of germination was observed at the lowest level (water content of only 29%).

According to Beltrame *et al.* (2018), considering different categories of orthodox, intermediate and recalcitrant seeds, with different levels of TD, requiring specific conditions to maintain physiological quality and ensure viability over time, turns indispensable.

Collar diameter (CD) of *P. roebelenii* seedlings at 65 days after sowing, decreased as a function of desiccation. Significant differences were observed in treatments in which seed water contents were under 18%, resulting in CD values between 1.72 and 1.85 cm, approximately. However, treatments with seed water contents above 18% (18-33%), led to non-significant CD values, ranging from 1.87 to 1.93 cm, (figure 3A, page 32).

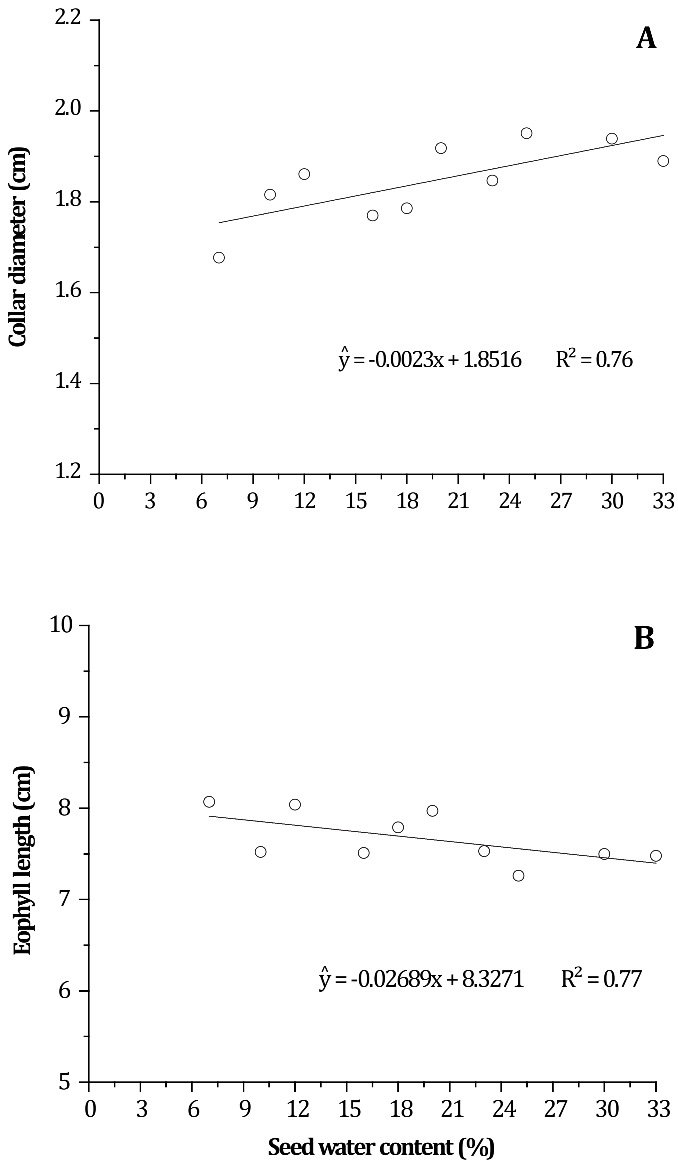


Significant at 5% ($p < 0.05$) probability.

Significativo al 5% ($p < 0,05$) de probabilidad.

Figure 2. Germination percentage (A) and germination speed index (B) of *Phoneix roebelenii* seeds as a function of seed water content.

Figura 2. Porcentaje de germinación de semillas (A) e índice de velocidad de germinación (B) de semillas de *Phoenix roebelenii* en función del contenido de agua de las semillas.



Significant at 5% ($p < 0.05$) probability.

Significativo al 5% ($p < 0{,}05$) de probabilidad.

Figure 3. Collar diameter (A) and eophyll length (B) of *Phoneix roebelenii* seedlings as a function of seed water content.

Figura 3. Diámetro del collar (A) y longitud del mesófilo (B) de plántulas de *Phoenix roebelenii* en función del contenido de agua de la semilla.

On the other hand, eophyll lengths of *P. roebelenii* seedlings were longer as seed water contents were reduced (figure 3B), and significantly higher fore seed water contents under 18%, varying from 7.87 to 8.15 cm (figure 3B).

The maximum storage time reached without seed viability loss is defined as longevity. In this context, seed longevity can be determined by the rate at which germination potential is lost over time, culminating with seed deterioration, that is seed capability of originating well formed and developed seedlings (17, 23). Therefore, it can be inferred that *P. roebelenii* seeds could maintain their viability, originating normal seedlings.

Studies involving physiological, genetic and biochemical mechanisms for abiotic stress tolerance (salinity and temperature) in *P. dactylifera* plants, guide cultivar development (10). According to Walters (2015), variations in seed response to storage and desiccation imply the existence of complex mechanisms and, bring about new technologies leading to understand viability regulation, ensuring seed availability.

Conclusions

P. roebelenii seeds are orthodox.

Reduced seed water content increased the percentage of germination, the germination speed index, and eophyll length of *P. roebelenii* seedlings.

Reduced seed water content did not affect first germination count, root length, root area, root diameter, root volume, and total fresh and dry weight of *P. roebelenii* seedlings.

Decreased seed water content resulted in reduced collar diameter of *P. roebelenii* seedlings.

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Glyphosate sprayed on the pre-existing vegetation reduces seedling emergence and growth of forage species

La aplicación de glifosato sobre la vegetación pre-existente reduce la emergencia y el crecimiento de las plántulas de especies forrajeras

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Abstract

Seeding pastures or forage crops by no-tillage methods usually involves the spray of glyphosate to suppress the existing vegetation. While many studies found detrimental effects of glyphosate on seed germination and seedling growth of the subsequent crop, others found negligible effects. This study aimed to evaluate the effect of glyphosate spraying on germination, seedling emergence and seedling growth of four forage species: *Trifolium repens*, *Lotus tenuis*, *Festuca arundinacea* and *Paspalum dilatatum*. The experiment was carried out spraying glyphosate on the pre-existing vegetation and on bare soil 1, 30, 60 and 90 days before sowing, and a control treatment sprayed with water. Glyphosate sprayed on pre-existing vegetation 1 to 60 days before seeding reduced emergence, while sprayed 1 to 30 or 1 to 60 days before seeding reduced seedlings belowground biomass and root length of all species and aboveground biomass of legumes respect to sprayed 90 days before seeding, sprayed on bare soil, and control treatment. This herbicide would remain active in the soil environment for at least 60 days after spraying when it was previously absorbed by plants, causing a severe damage to seedlings emergence and growth.

Keywords
herbicide • germination • legumes • grass • vegetation cover • bare soil • elapsed-time

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Resumen

La siembra directa de pasturas o verdeos involucra la pulverización con glifosato para eliminar la vegetación pre-existente. Si bien algunos estudios encontraron que el glifosato provocó efectos negativos en la germinación y el crecimiento de plántulas del cultivo, otros no encontraron efectos significativos. El objetivo de este trabajo fue evaluar el efecto de la pulverización con glifosato sobre la germinación y el crecimiento de las plántulas de cuatro especies forrajeras: *Trifolium repens, Lotus tenuis, Festuca arundinacea* y *Paspalum dilatatum*. Se pulverizó glifosato sobre la vegetación o sobre suelo desnudo 1, 30, 60 o 90 días antes de la siembra y se pulverizó con agua al tratamiento control. El glifosato pulverizado sobre la vegetación 1 a 60 días antes de la siembra redujo la emergencia, pulverizado 1 a 30 o 1 a 60 días antes de la siembra redujo la biomasa subterránea y la longitud de raíces de todas las especies y la biomasa aérea de las leguminosas respecto de la pulverización 90 días antes de la siembra, sobre el suelo desnudo y del tratamiento control. Este herbicida se habría mantenido activo en el suelo durante al menos 60 días luego de la pulverización, cuando fue previamente absorbido por la vegetación, causando un severo daño en la emergencia y el crecimiento de las plántulas.

Palabras clave
herbicida • germinación • leguminosas • pastos • cobertura vegetal • suelo desnudo • tiempo de carencia

Introduction

Glyphosate is a non-selective broad-spectrum herbicide, widely used on global scale (3, 12). The extended use of glyphosate has been attributed to its efficacy on control weed species (26), but an increasing number of reports suggest negative side effects on non-target plants (5). Many studies have found detrimental effects of glyphosate on seed germination and seedling growth of several crop species (1, 4, 8, 28, 31). However, the results are not conclusive because other studies have found little or negligible effects on crop species (7, 18, 27).

In cattle or sheep grazing systems, glyphosate is sprayed to suppress the existing vegetation before seeding or reseeding pastures or forage crops by no-tillage methods as direct drilling or broadcasting seeds (2, 34). However, little information is available about the effect of glyphosate on seed germination and seedling growth of forage species because most studies about the exposure of glyphosate on the root-zone were carried out on crop species and rarely in a real soil substrate.

Numerous studies consider that glyphosate toxicity to non-target plants in soils is marginal because it is inactivated by microbial degradation and adsorption by soil constituents (3). Glyphosate is a polar compound which adsorbs strongly to soil iron and aluminum oxides and clays (24). Therefore, the adsorption of glyphosate and aminomethylphosphonic acid (AMPA) increased strongly with iron and aluminum content of soils and decreased with increasing soil organic carbon content. This would indicate that glyphosate and AMPA are mainly adsorbed by clay minerals, while soil organic matter competes for adsorption sites and inhibits adsorption (14). Such soil binding is reversible, and after desorption glyphosate is degraded by various bacteria to AMPA, its main secondary metabolite, and ultimately to inorganic phosphate, ammonia and carbon dioxide (3, 4). Therefore, the labile pool of glyphosate in soils to be absorbed by plant roots could be dramatically reduced and, glyphosate toxicity over non-target plants would be negligible (15). Supported by these evidences, it was globally widespread that glyphosate is inactive in the soil, and therefore its phytotoxic effect could be considered as negligible. However, other studies detected residues of glyphosate and AMPA in soil solution after six months after spraying (32). A similar degradation process of glyphosate to AMPA also occurred in some plant species (29), a portion of these residues in soil would come from the glyphosate absorbed by plants, translocated towards the roots and underground organs and then exuded to the surrounding solution (21). Therefore, an important source of soil glyphosate or AMPA is root exudation from sprayed plants and its release from dead plants, which implies a toxicity risk to non-target plants due to rhizosphere transfer (16).

Due to their chemical similarities, both glyphosate and AMPA can be taken up by roots from the soil solution (16), so affecting the growth of seedlings. In forage species, only one report stated that the incorporation of glyphosate before sowing into a *Vertic Argiudol* reduced chlorophyll content and above and belowground growth in seedlings of the legume *Lotus corniculatus* (8). In accordance, there is evidence that rootlets of soybean and maize seeds that germinate after spraying could absorb secondary metabolites of glyphosate, mainly AMPA exuded by roots of sprayed plants (31). Besides, glyphosate degradation resulted two to six times slower when it was previously absorbed by plants than when it was directly applied to the soil (10). The elapsed-time between glyphosate spraying and sowing was directly related to sunflower seedling growth and biomass production, and glyphosate had yet detrimental effects at the largest elapsed-time of 21 days (33). Glyphosate markedly inhibited growth of rootlets and shoots of *Raphanus sativus* seedlings exposed to the herbicide in the root-zone after 7 and 14-days of exposure (18). Negative effect on seed germination was found when seeds of legume species were in direct contact with glyphosate in water solution (17, 23). In summary, contrary to the idea that glyphosate is inactivated in soil, several studies suggest that glyphosate spraying on the pre-existing vegetation may cause deleterious effects on seed germination, seedling emergence and growth of the subsequent crop.

Therefore, the aim of this study was to evaluate the effect of glyphosate spraying on germination, seedling emergence and seedling growth of four greatly extended forage species. We predicted that negative effects of glyphosate on seed germination and establishment would be greater when glyphosate is sprayed on pre-existing vegetation than on bare soil, and that the elapsed-time between spraying and seeding will be positively related to germination, seedling emergence and growth of forage seedlings.

Materials and methods

Experimental design

We extracted soil from paddocks of a livestock farm located in the center of the Flooding Pampa region (36°40'12" S, 59°32'10" W, 80 m a. s. l.). We selected paddocks covered by native grasslands that had never been sprayed with any herbicide. The dominant soil belongs to the series General Guido, *typic Natraquoll*, characterized by an acidic (pH 6.8), non-saline A_1 horizon 12 cm-depth and a clayey and sodic B_{2t} horizon (pH 8.2). Soil was extracted from the top horizon, sieved to remove vegetation and seeds, and then distributed in 400 plastic containers of 13 x 17 x 25 cm. Containers were maintained in a greenhouse with controlled temperature (20-25°C), natural light and regular watering throughout the experimental period. In April 2010, 20 seeds of each forage species *L. tenuis*, *T. repens, P. dilatatum* and *F. arundinacea* were sown in 100 containers per species and covering them with a thin layer of the same soil.

To compare the effects of glyphosate sprayed on pre-existing vegetation cover (C+) or on bare soil (C-) on germination, seedling emergence and seedling growth of the four forage species, we generated a vegetation cover of *Lolium multiflorum* Lam plants. For this, we harvested seeds of *L. multiflorum* in December 2009 from the same grassland paddocks where soil was extracted. Twenty-five seeds of *L. multiflorum* per container were sown in 200 containers 45 days before glyphosate spraying. Plants of *L. multiflorum* were thinned to 25 plants per container and periodically cut to maintain a similar height of 12 cm. In the 200 containers under C+ level, *L. multiflorum* plants were cut to ground level two days after sowing to avoid confounding effects of competition between *L. multiflorum* plants and forage seedlings. Other 200 containers were not sown with *L. multiflorum* to obtain the C- level.

To evaluate whether the elapsed-time between glyphosate spraying and sowing was positively related to germination, seedling emergence and growth of seedlings, glyphosate was sprayed 1 (G1), 30 (G30), 60 (G60) and 90 (G90) days before sowing the forage seeds or was not sprayed (G0 - control treatment sprayed with water 1day before sowing) (9).

A monoammonium salt of glyphosate (1440 g ae ha^{-1}) was applied at a dose of 2.88 L of glyphosate ha^{-1} with a CO_2 pressurized backpack sprayer fitted with a TT11001 nozzle delivering 100 L ha^{-1} at 200 kPa (36). The levels G1, G30, G60 and G90 consisted in 40 containers that were kept without vegetation cover (C-) and 40 containers that were sown with 25 seeds of *L. multiflorum* (C+) 60 days before glyphosate spraying. Control level G0 consisted in 80 containers (40 C+ and 40 C-) sprayed with the same amount of water without glyphosate one day before the sowing of forage seeds.

In summary, a completely randomized factorial design with 5 replications was performed. The two main factors were 1) Cover on which glyphosate was sprayed (C), at 2 levels: presence (C+) or absence (C-) of vegetation cover; and 2) Spraying of glyphosate before sowing (G), at 5 levels: sprayed 1(G1), 30 (G30), 60 (G60) and 90 (G90) days before sowing or not sprayed (G0). The same experiment was performed for each forage species and we analyzed the responses of each forage species to glyphosate treatments but did not compare among species due the great morphological and physiological differences among them.

Plant material

We selected two forage legume species, *Lotus tenuis* Waldst. cv. Aguapé and Kit. Ex Willd and *Trifolium repens* L. *cv.* El Lucero and two forage grass species *Paspalum dilatatum* Poir. (cv. Relincho) and *Festuca arundinacea* (Schreb.). These species are worldwide used in mixed pastures and are also naturalized in native grasslands of temperate regions (13). *T. repens* and *F. arundinacea* grow during the cool-season period, while *L. tenuis* and *P. dilatatum* grow during the warm-season period. Seeds of *L. tenuis*, *T. repens*, *P. dilatatum* and *F. arundinacea* were provided by commercial seed breeders.

Response variables

The number of emerged seedlings was daily recorded until twenty consecutive days without new emergencies. This period averaged 40 days after sowing. After 60 days from sowing, all seedlings were carefully harvested and seeds remaining in the soil were exhumed by sieving the soil through two sieves of 2.0 and 0.2 mm and examined under an optical microscope to determine whether germination had initiated (*i.e.* rootlet appearance) (30). The exhumed seeds that did not germinate (*i.e.* without visible rootlet) were evaluated by the tetrazolium test to determine their viability (19). The response variables related to seed germination and seedling emergence were expressed in percentage and calculated as following: a) Seed germination (%) as the number of established seedlings plus the number of exhumed germinated seeds in relation to the number of seeds sown of each species, b) Viable seeds (%) as the number of established seedlings plus the number of exhumed germinated seeds plus the number of viable not germinated seeds in relation to the number of seeds sown of each species, and Seedling emergence (%) as the number of established seedlings in relation to the number of seeds sown of each species (30).

Seedling morphological traits were assessed after seedling removal by measuring the number of leaves of the unique tiller and the length of the third leaf (lamina plus sheath) in the grasses *P. dilatatum* and *F. arundinacea*, the number and average length of the stems in *L. tenuis* and the number of leaves and the average length of the petioles in *T. repens.* The length of the seminal root of the four species was also measured. All length measurements were carried out by using a mechanical caliber. Aboveground and belowground biomass of seedlings were determined by oven drying until constant weight.

Statistical analyses

Variables were analyzed separately for each species through a factorial two-way ANOVAs at $\alpha=0.05$, being the main factors C and G. Tukey tests were applied to *post-hoc* comparison among means. Variables that involve percentages (seed germination, seedling emergence and viable seeds) were arcsine square-root transformed before analysis to meet ANOVA assumptions. Normality and homogeneity of variances were previously checked performing Kolmogorov-Smirnov and Levene and Brown-Forsythe tests. The results that involve percentages are shown as non-transformed mean $\pm$ 1 SD. Statistical analyses were performed using the package STATISTICA for Windows (StatSoft,Tulsa, OK, USA).

Results

Effects of glyphosate spraying on seed germination and viability

Germination and seed viability were not affected by the main factors C and G or their interaction. Germination was 50.6 ±12.3% for *L. tenuis*, 57.3±5.9 % for *T. repens*, 77.3±13.0% for *P. dilatatum* and 77.1±11.5% for *F. arundinacea*, while seed viability was 84.96±7.37% for *L. tenuis*, 82.16±6.28% for *T. repens*, 87.69±6.81% for *P. dilatatum* and 90.17±3.25% for *F. arundinacea*.

Effects of glyphosate spraying on seedling emergence

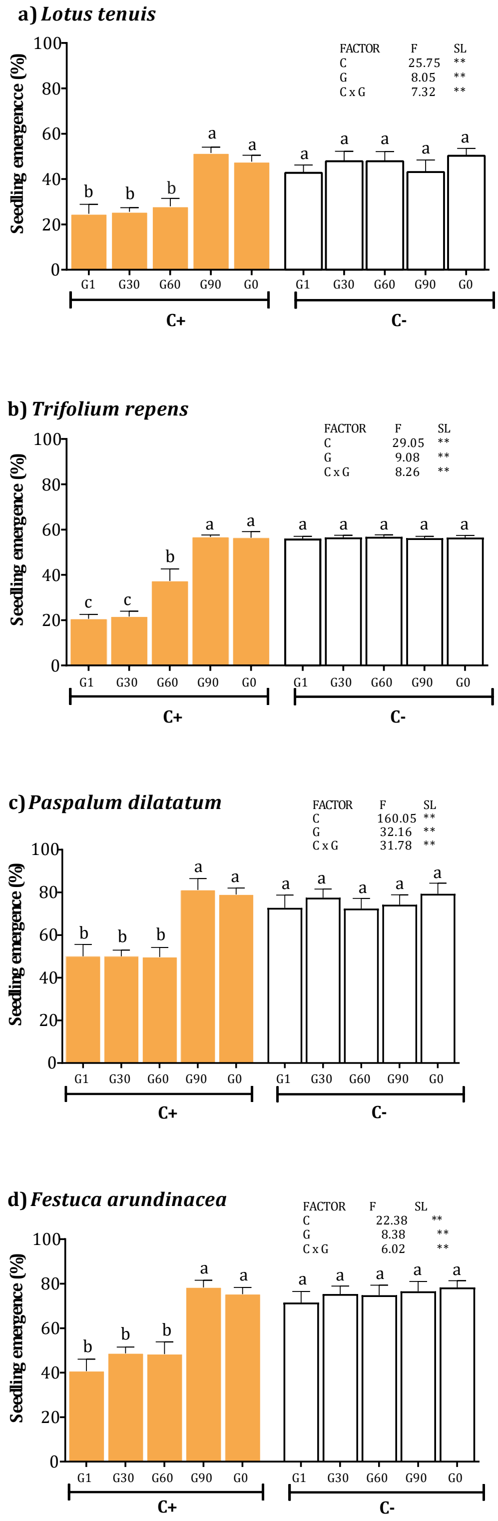
Seedling emergence of the four forage species was clearly affected by the interaction between them. Seedling emergence showed a consistent pattern among species: it was lower under C+ at G1, G30 and G60, than under C+ at G90 and G0 or under C- at any G level (figure 1, page 40). Therefore, glyphosate sprayed on preexisting vegetation cover 1 to 60 days before sowing caused a negative effect on seedling emergence of different magnitude depending on the species. Seedling emergence of *L. tenuis* under C+ at G1, G30 and G60 was 45% lower than under C+ at G90 and G0 and C- at G1, G30, G60, G90 and G0 (26.1+10% *vs*. 47.4+11.5% respectively figure 1 a, page 40). *T. repens* showed the lowest seedling emergence under C+ at G1 and G30 (21.1±6.9%), which was almost 63% lower than under C+ at G90 and G0 and C- at any G levels (56.5±3.7%) (figure 1 b, page 40). Seedling emergence of *P. dilatatum* under C+ at G1, G30 and G60 was 35% lower than under C+ at G90 and G0 and C- at any G levels (49.9±13.9%vs 76.6±14.8% respectively, figure 1 c, page 40). Seedling emergence of *F. arundinacea* under C+ at G1, G30 and G60 was almost 40% lower than under C+ at G90 and G0 and C- at any G levels (45.9 ±14.5% *vs*. 75.7±12.1% respectively figure 1 d, page 40).

Effects of glyphosate on seedling biomass

Above and belowground biomass of legumes seedlings was affected by the main factors C and G or by the interaction between them. Under C+ at G1 and G30, aboveground biomass of *L. tenuis* was 83% lower and above and belowground biomass of *T. repens* seedlings was 51% lower than under C+ at G90 and G0 and under C- at any G level (0.0169 ±0,0069 g. *vs*. 0.0987±0.0393 g; 0.0105±0.0047 *vs*. 0.0213 ±0.0054 g and 0.0067±0,0037 *vs*. 0.0136±0.0042 g respectively, figure 2 a and b, page 41). Belowground biomass of *L. tenuis* seedlings was lower under C+ than under C- and at G1 and G30 respect to G90 and G0 (figure 2 a, page 41). Under C+ at G60, aboveground biomass of *L. tenuis* seedlings was higher than under C+ at G1 and G30 but lower than under C+ at G90 and G0 and under C- at any G level (figure 2 a, page 41), while above and belowground biomass of *T. repens* seedlings did not differ respect to the other G levels (figure 2 b, page 41). Belowground -but not aboveground- biomass seedling of grasses was also affected by the main factors C and G and by the interaction between them. Belowground biomass of *P. dilatatum* seedlings was 46% lower under C+ at G1 and G30 than under C+ at G90 and G0 and under C- at any G level (0.0122±0.0050 g *vs*. 0.0224 ± 0.0049 g, (figure 2 c, page 41). Belowground biomass of *F. arundinacea* was 40% lower under C+ at G1 and G30 than under C+ at G60, G90 and G0 and C- at any G level (0.0148 ±0.0033 g *vs*. 0.0248±0.0047 g, (figure 2 d, page 41).

Effects of glyphosate spraying on morphological variables

Morphological variables of legumes seedlings were affected by the main factors C and G and by the interaction between them (figure 3, page 42). The number of stems of *L. tenuis* (figure 3 a, page 42), the petiole length of *T. repens* (figure 3 c, page 42) and the root lengths of both species (figure 3 b and figure 3 d, page 42) were significantly reduced under C+ at G1 and G30 respect to under C+ at G90 and G0 and under C- at any G level. The number of leaves per tiller and the leaf length of grasses were not affected by any treatments, but the root length of both grasses *P. dilatatum and F. arundinacea* was lower under C+ at G1 and G30 respect to under C+ at G60, G90 and G0 and under C- at any G level (figure 3 e and figure 3 f, page 42).

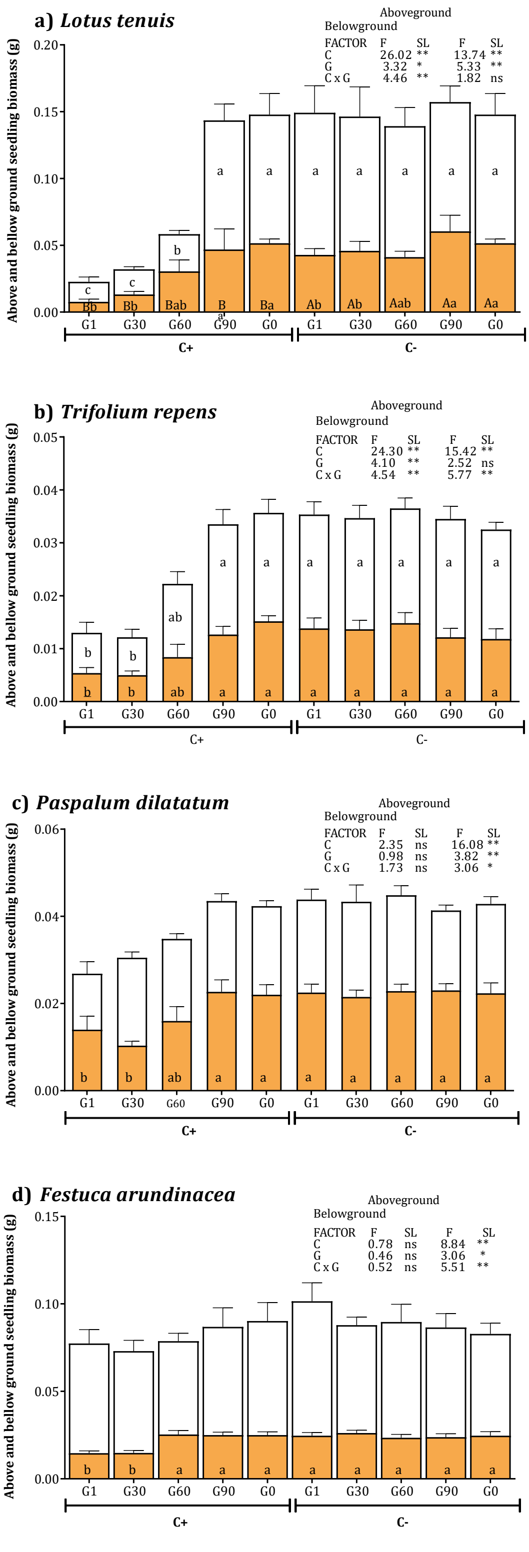


Vertical bars indicate 1 SD of the means. Different letters indicate significant differences provided by the Tukey test of the interaction factor (P< 0.05). Insert, results of ANOVA test: Main factors are Cover on which glyphosate was sprayed (C, at two levels C+ and C-) and Spraying of glyphosate before sowing (G, at five levels G1, G30, G60, G90 or G0). Significant levels (SL) are indicated with an asterisk (*) when P<0.05 and with two asterisks (**) when P<0.01.

Las barras verticales indican 1 desvío estándar de las medias. Letras distintas indican diferencias significativas según el test de Tukey (P<0,05) para el factor interacción. Se insertaron los resultados del ANOVA: Los factores principales son Cobertura sobre la que se pulverizó glifosato (C, a dos niveles: C+y C-) y Pulverización de glifosato antes de la siembra (G, a cinco niveles: G1, G30, G60, G90 or G0). El nivel de significancia (SL) se indica con un asterisco (*) cuando P<0,05 y con dos asteriscos (**) cuando P<0,01.

Figure 1. Seedling emergence (%) of *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) and *Festuca arundinacea* (d) subjected to glyphosate spraying on the preexisting *L. multiflorum* vegetation cover (C+; orange bars) or bare soil (C-; white bars) at 1, 30, 60, 90 days before sowing and a control treatment not sprayed (G1, G30, G60. G90 and G0).

Figura 1. Emergencia de plántulas (%) de *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) y *Festuca arundinacea* (d) pulverizadas con glifosato sobre la vegetación pre-existente de *L. multiflorum* (C+; barras naranjas) o sobre suelo desnudo (C-; barras blancas) 1, 30, 60, 90 días antes de la siembra y el tratamiento control no pulverizado (G1, G30, G60, G90 y G0).



Vertical bars indicate 1 SD of the means. Different letters indicate significant differences provided by the Tukey test of the interaction factor (P< 0.05). Insert, results of ANOVA test: Main factors are Cover on which glyphosate was sprayed (C, at two levels C+ and C-) and Spraying of glyphosate before sowing (G, at five levels G1, G30, G60, G90 or G0). Significant levels (SL) are indicated with an asterisk (*) when P<0.05 and with two asterisks (**) when P<0.01.

Las barras verticales indican 1 desvío estándar de las medias. Letras distintas indican diferencias significativas según el test de Tukey (P< 0,05) para el factor interacción. Se insertaron los resultados del ANOVA: Los factores principales son Cobertura sobre la que se pulverizó glifosato (C, a dos niveles: C+y C-) y Pulverización de glifosato antes de la siembra (G, a cinco niveles: G1, G30, G60, G90 or G0). El nivel de significancia (SL) se indica con un asterisco (*) cuando P<0,05 y con dos asteriscos (**) cuando P<0,01.

Figure 2. Above (white bars) and belowground (orange bars) biomass of seedlings of *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) and *Festuca arundinacea* (d) subjected to glyphosate spraying on the preexisting *L. multiflorum* vegetation cover (C+; orange bars) or bare soil (C-; white bars) at 1, 30, 60, 90 days before sowing and a control treatment not sprayed (G1, G30, G60, G90 and G0).

Figura 2. Biomasa aérea (barras blancas) y subterránea (barras naranjas) de las plántulas de *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) y *Festuca arundinacea* (d) pulverizadas con glifosato sobre la vegetación pre-existente de *L. multiflorum* (C+; barras naranjas) o sobre suelo desnudo (C-; barras blancas) 1, 30, 60, 90 días antes de la siembra y el tratamiento control no pulverizado (G1, G30, G60, G90 y G0).

Vertical bars indicate 1 SD of the means. Different letters indicate significant differences provided by the Tukey test of the interaction factor (P< 0.05). Insert, results of ANOVA test: Main factors are Cover on which glyphosate was sprayed (C, at two levels C+and C-) and Spraying of glyphosate before sowing (G, at five levels G1, G30, G60, G90 or G0). Significant levels (SL) are indicated with an asterisk (*) when P<0.05 and with two asterisks (**) when P<0.01.

Las barras verticales indican 1 desvío estándar de las medias. Letras distintas indican diferencias significativas según el test de Tukey (P< 0,05) para el factor interacción. Se insertaron los resultados del ANOVA: Los factores principales son Cobertura sobre la que se pulverizó glifosato (C, a dos niveles: C+y C-) y Pulverización de glifosato antes de la siembra (G, a cinco niveles: G1, G30, G60, G90 or G0). El nivel de significancia (SL) se indica con un asterisco (*) cuando P<0,05 y con dos asteriscos (**) cuando P<0,01.

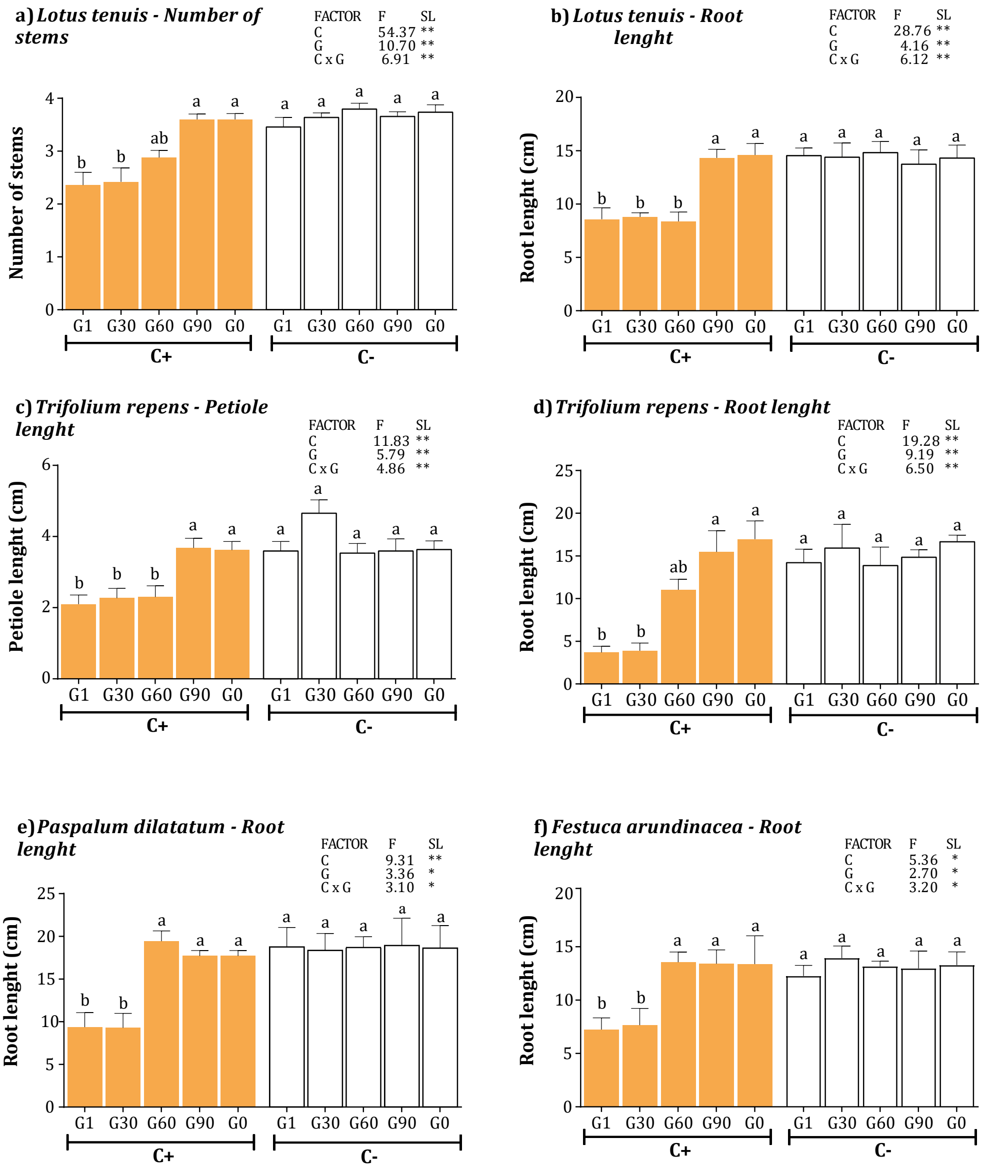


Figure 3. Number of stems (a) and root lenght (b) of *Lotus tenuis*, petiole length (c) and root length (d) of *Trifolium repens*, root lenght of *Paspalum dilatatum* (e) and root length of *Festuca arundinacea* (f) subjected to glyphosate spraying on the preexisting vegetation of *L. multiflorum* cover (C+; orange bars) or bare soil (C-; white bars) at 1, 30, 60, 90 days before sowing and a control treatment not sprayed (G1, G30, G60, G90 and G0).

Figura 3. Cantidad de tallos (a) y longitud de raíces (b) de *Lotus tenuis*, longitud de pecíolos (c) y longitud de raíces (d) de *Trifolium repens*, longitud de raíces of *Paspalum dilatatum* (e) y longitud de raíces de *Festuca arundinacea* (f) pulverizadas con glifosato sobre la vegetación pre-existente de *L. multiflorum* (C+; barras naranjas) o sobre suelo desnudo (C-; barras blancas) 1, 30, 60, 90 días antes de la siembra y el tratamiento control no pulverizado (G1, G30, G60, G90 y G0).

Discussion

Our results showed that glyphosate spraying on preexisting vegetation cover 1 to 60 days before sowing reduces seedlings emergence and growth of the forage legumes *Lotus tenuis* and *Trifolium repens*, and the forage grasses *Paspalum dilatatum* and *Festuca arundinacea*. The negative effect showed a threshold response because it prevailed during 30 or 60 days since glyphosate spraying until sowing, depending on the variable and the forage species.

We found that glyphosate greatly reduced seedling emergence and seedling growth when it was sprayed on pre-existing vegetation but not when it was sprayed on bare soil. Glyphosate mineralization rate in soil is much lower when it was previously absorbed by plants than when it is applied to bare soil (10, 21). Herbicides trapped in plant residues provides protection against microbial degradation (21), so glyphosate degradation in soil following their absorption by plants is much slower, which increase it persistence from two to six times respect to directly sprayed on bare soil (10). Glyphosate or AMPA are released from tissues of treated plants to the rhizosphere zone and transfer to non-target plants via contact contamination (5, 25, 33). On the contrary, when glyphosate is sprayed on bare soil, it suffers a rapid microbial degradation or inactivation by sorption to the soil matrix (15).

Our results indicated that the reduction of seedling emergence when glyphosate was sprayed on pre-existing vegetation can be attributed to the toxic effect on germinated seeds, after rootlet appearance, because seed germination and seed viability were not affected. The mortality of germinated seeds occurred before seedling emergence, suggesting a greater susceptibility to the herbicide in younger tissues and seedlings of small size, susceptibility that decreases as the size or the state of development of the plants increases (20, 35). Contrary to the studies that found reduction of seed germination when seeds germinated on boxes on filter paper and watered with glyphosate-water solutions (17, 22), seed germination was not affected when glyphosate was sprayed on vegetation cover or bare soil, not directly over the seeds.

Seedlings that survived to glyphosate sprayed on the pre-existing vegetation, suffered a decline of its growth. Both legume and grass seedlings showed lower belowground biomass and root length, and seedlings of legumes also showed much lower aboveground biomass. These are indirect symptoms, mainly expressed as limitations to root functioning like absorption of water (38) and nutrients (25), which result in lower above and belowground growth. Moreover, our results suggest higher susceptibility to glyphosate of legumes than grasses, as species, and even biotypes of the same species, differ in their susceptibility to glyphosate (3, 6).

Glyphosate persistence in soil is extremely variable as it is affected by soil properties, method of application, and environmental conditions, such as moisture and temperature (11). Our experiment showed that, in a *typic Natracuol* soil, the deleterious effect of glyphosate on seedling emergence and seedling growth had a threshold response pattern as it prevailed during at least 30 or 60 days between glyphosate spraying and sowing, with similar intensity along this period. Therefore, the recommendation of spraying glyphosate 7-30 days before seeding or overseeding pastures and forage species by no-tillage methods (1, 2, 37) would not be appropriate in order to avoid injury on seed emergence and seedling growth.

Conclusion

Our results suggest that the elapsed-time between glyphosate spraying on pre-existing vegetation of *L. multiflorum* and sowing of *L. tenuis*, *T. repens*, *P. dilatatum* and *F. arundinacea* seeds in argillic and natric soils must be longer than 60 days to avoid a reduction of seedling emergence and to maximize seedlings size and biomass. This finding is relevant for management purposes because of the prevalence of argilic and natric soils such as *Argiudols* and *Natracuols* in the Pampa region, where seeding or overseeding pastures and annual forage crops by no-tillage methods after glyphosate spraying is an extended practice.

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Impact of paclobutrazol on gibberellin-like substances and soluble carbohydrates in pear trees grown in tropical semiarid

Impacto del paclobutrazol en sustancias similares a la gibberelina y carbohidratos solubles en peras cultivadas en el semiárido tropical

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Abstract

Given that Brazilian pear production is unable to meet the annual demand, to consider the possible expansion to non-traditional growing regions, turns interesting. Even though under tropical semi-arid conditions pear trees have vigorous vegetative growth, a negative influence on flower bud differentiation and formation affects fruit yield. Our study aimed to evaluate the inhibition efficiency of paclobutrazol (PBZ) on gibberellin biosynthesis, vegetative growth, and carbohydrate production in two pear-tree cultivars ('Santa Maria' and 'Hosui') grown under semi-arid conditions. To this end, two experiments were conducted, one for each pear-tree cultivar. The experimental designs consisted of randomized blocks, with factorial arrangement (5x2x4), corresponding to PBZ doses (0.0, 0.5, 1.0, 1.5, and 2.0 g per linear meter of plant canopy), PBZ application forms (soil and foliar), and evaluation dates (30, 60, 90, and 120 days after application). Both soil and foliar applications inhibited gibberellin biosynthesis in both cultivars, especially after 120 days of application. PBZ affected leaf total soluble carbohydrates and reduced sprout growth in both cultivars. Although PBZ can be potentially used in pear management, further studies are still required to determine specific management practices in tropical semi-arid zones.

Keywords
Pyrus sp. • growth regulator • gibberellic acid • PBZ

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Resumen

Debido a que en Brasil, la producción de peras es reducida en relación con la demanda anual, la expansión del cultivo a regiones no tradicionales es esencial. En condiciones tropicales semiáridas, el peral presenta un crecimiento vegetativo vigoroso que influye negativamente en la diferenciación floral, en el desarrollo de yemas florales y en consecuencia, en el rendimiento de frutos. Este estudio tuvo como objetivo evaluar la eficiencia del paclobutrazol (PBZ) en la inhibición de la biosíntesis de giberelinas y en la producción de carbohidratos en cultivares de pera 'Santa Maria' y 'Hosui', en condiciones semiáridas. Se realizaron dos experimentos, uno para cada cultivar. El diseño experimental consistió en bloques al azar, con arreglo factorial (5x2x4) según dosis del PBZ (0,0; 0,5; 1,0; 1,5 y 2,0 g por metro lineal de canopia), forma de aplicación del PBZ (edáfica o foliar) y fechas de evaluación (30, 60, 90 y 120 días después de la aplicación del PBZ). La aplicación de PBZ a través del suelo o las hojas inhibe la biosíntesis de giberelinas en los cultivares de pera 'Santa Maria' y 'Hosui', afectando los niveles de carbohidratos solubles en hojas totales y reduce el crecimiento de brotes para ambos cultivares. Aunque el PBZ se podría utilizar en el manejo del peral, se requieren más estudios para determinar prácticas de manejo específicas en zonas tropicales semiáridas.

Palabras clave
Pyrus sp. • regulador del crecimiento • ácido giberélico • PBZ

Introduction

Pear (*Pyrus* sp.) belongs to the Rosaceae family, comprising more than twenty species of European (*Pyrus communis* L.) and Asian (*Pyrus pyrifolia*) origins (27). It is worldwide grown and appreciated, mainly for fresh fruit consumption (12, 13). In Brazil, pear cultivation is concentrated in the southern region. However, production is small in relation to the Brazilian demand, and raising imports may reach up to 90% of the total demand (18).

The São Francisco Valley is considered one of the main fresh fruit producing and exporting regions in Brazil, with an increasing production potential, mainly given by market harvest windows that differ from those of traditional producing regions. Therefore, fruits can be made available at times of high market demand (26, 34).

The São Francisco Valley is located in a semi-arid tropical region. In this condition, pear trees growing under high temperatures and constant irrigation do not stop their metabolic functions during the cycle, accelerating branching (25). Vigorous shoot growth impedes light from reaching the inside of canopies, affecting differentiation and formation of flower buds (15), and hence fruit yield and quality. In this sense, controlling vegetative growth turns essential for profitable management. In young orchards, this control can anticipate flowering and fruiting, whereas in adult orchards it avoids shading and favors fruit production (8). Viewed in this way, the use of plant growth regulators to inhibit gibberellin biosynthesis, plant hormones responsible for branch and shoot elongation (5), can be an alternative to improve vegetative *vs.* reproductive growth.

Among the most used plant growth regulators for vigor control, paclobutrazol (PBZ) has shown high efficiency. PBZ is a triazole derivative that inhibits oxidation of *ent*-kaurene to *ent*-kaurenoic acid during gibberellin biosynthesis (31), when plant cell elongation and division are induced. Besides controlling vegetative growth, PBZ stimulates rooting and increases chlorophyll levels, carbohydrate concentrations, cytokinin synthesis, and abscisic acid (27, 33). This regulator can be absorbed through roots, branches, and foliage (3).

This study aimed to evaluate the inhibition efficiency of PBZ on gibberellin biosynthesis, vegetative growth, and carbohydrate production in 'Santa Maria' and 'Hosui' pear-tree cultivars, grown under semi-arid conditions.

Material and methods

The study included 120 'Hosui' and 'Santa Maria' pear trees (*Pyrus* sp.) grafted onto *Pyrus calleryana* L. and transplanted in 2013. The study was conducted from March 2017 to February 2018 on an experimental orchard located in Serenissima farm, in Lagoa Grande (09°21' S and 40°34' W; at an altitude of 375 m above sea level), Pernambuco State, Brazil. The regional climate is classified as Bsh (Köeppen), corresponding to a semiarid region.

The orchard was conducted with a central leader with 4.0 m between rows, and 1.25 m between trees. The trees were drip-irrigated daily with ten self-regulated emitters per tree with a 2 L $hour^{-1}$ flow based on daily evapotranspiration recorded by the Embrapa Meteorological Station and corrected according to apple Kc. Fertilizing management was performed according to soil analysis, while other cultural practices were performed according to Quezada *et al.* (2003).

Two independent experiments were performed for each pear cultivar. The experimental design consisted of randomized blocks with factorial arrangement (5 x 2 x 4). Treatments were paclobutrazol (PBZ) doses (0.0, 0.5, 1.0, 1.5 and 2.0 g per linear meter of plant canopy), PBZ application form (soil or foliar spray) and evaluation dates (30, 60, 90 and 120 days after PBZ application), with four replications and three plants per parcel. PBZ doses were defined according to those indicated for mango (*Mangifera indica*) since no recommendation for pear is available to the date. 'Santa Maria' and 'Hosui' pear trees were pruned and once the first uniform shoots (5 cm length) were visible, the treatments were applied on March 24th, 2018 and April 26th, 2018, respectively.

For soil-treated plants, each PBZ dose was diluted in 2 L of water and applied at a distance of 50 cm from the plant stem. For leaf treatments, plants were sprayed with each PBZ dose until complete wetting. For both application forms, each treatment was applied once, following the recommendations of Genú and Pinto (2002) using as PBZ source Cultar SC® (25% a.i.).

All variables were recorded from the beginning of the experiments (treatments application) and later at 30, 60, 90, and 120 days after treatments establishment. Shoot length (cm) and diameter (mm) were measured in sprouts from the middle of the canopy. Total soluble carbohydrates (LSC) were quantified in completely expanded leaves from the middle part of the canopy and following the methodology described by Dubois *et al*. (1956).

Total GA concentrations were determined and quantified in flower buds at 30, 60, 90, and 120 days after treatments. This last date coincided with flower induction phase. The collected flower buds were immediately immersed in liquid nitrogen and taken to the UNIVASF Plant Physiology Laboratory. Once at the laboratory, 50 mg of each previously macerated sample, were vortexed in Eppendorf tubes (A) with 1.0 ml of 80% methanol, for 30 seconds. Then, taken to the ultrasonic bath for 5 minutes and immediately centrifuged at 13,000 rpm for 10 minutes. The supernatant was removed with a pipette and transferred to a second Eppendorf (B) for the first extraction. Subsequently, the second and third extractions were performed on Eppendorf A, adding 0.5 ml of 80% methanol. Later, the supernatant was removed from Eppendorf B, obtaining a single solution. Finally, the extracts were filtered through a 0.45 µm syringe filter and taken to the ultrasonic bath for 3 minutes before chromatography. The calibration curve was obtained by subsequent measurements of increasing dilutions from a standard GA_3 stock solution (Sigma-Aldrich®) ranging from 2.5 - to 50 µg/ml. Each dilution was filtered through a 0.45 µm syringe filter and sonicated for 5 min. Then, 20 µL were injected in the chromatograph Shimadzu® LC-20 model.

Total GAs were determined with a liquid chromatograph using C-18 column (SUPELCO 150 x 4.6 mm, 5 µm, Ascentis® C18, Phenomenex®), with a ratio of 40:60 (A/B) phase referring to 0.1% formic acid in ultrapure water and methanol, respectively, in an isocratic flow 1 ml/min. Twenty µl samples were injected and monitored at 206 nm, according to Macías *et al*. (2014) with modifications. Identification was made by comparing retention times and spectrum with the standard. The data obtained were analyzed using Shimadzu® LC solution 1.0 software (Japan). Total GAs concentrations were expressed as µg of gibberellic acid (EGA_3) per gram of sample (fresh mass) according to the total UV peak area detected by the chromatograph.

Data were submitted to analysis of variance (ANOVA). Statistical analyses were performed using SISVAR and SIGMAPLOT softwares. Differences were considered significant with p <0.05, except for total gibberellins, for which only one sample per treatment was available, and analyzed as composite samples. PBZ doses were submitted to regression analysis using R software (30).

Results and discussion

Table 1 shows a triple interaction effect on leaf total soluble carbohydrates (LSC) for cv. Santa Maria. The plants underwent physiological and biochemical changes throughout the cycle, with increases in LSC contents.

Table 1. Sprout length (SL), sprout diameter (SD) and leaf soluble carbohydrates (LSC) of 'Santa Maria' and 'Hosui' pear cultivars as a function of PBZ levels (L), application form (F) and evaluation dates (D).

Tabla 1. Longitud del brote (SL), diámetro del brote (SD) y carbohidratos solubles en hojas (LSC) de los cultivares de pera 'Santa Maria' y 'Hosui' en función de los niveles del PBZ (L), forma de aplicación (F) y fechas de evaluación (D).

Variation source	SL (cm)		SD (mm)		LSC (μmol.g^{-1} FM)	
Appl. Form (F)	'Santa Maria'	'Hosui'	'Santa Maria'	'Hosui'	'Santa Maria'	'Hosui'
	5.01 *	11.69**	3.62^{ns}	52.05**	1.79^{ns}	0.25^{ns}
Soil	27.91a	18.71a	5.97a	6.27a	115.76a	123.34a
Leaf	31.43b	22.73b	6.19a	6.96b	122.79a	120.61a
MSD	3.11	2.32	0.23	0.18	10.39	10.64
PBZ level (L)	261.02**	244.46**	159.08**	247.14**	0.71^{ns}	2.36^{ns}
Dates (D)	10.05**	17.38**	27.76**	58.99**	5.14**	29.07**
L x F	32.50**	18.66**	12.78**	11.74**	2.36^{ns}	1.37^{ns}
F x D	0.03^{ns}	0.50^{ns}	0.98^{ns}	2.02^{ns}	17.45**	6.06**
L x D	3.77**	3.84**	5.27**	5.78**	1.68^{ns}	1.92*
L x F x D	0.27^{ns}	0.04^{ns}	0.54^{ns}	0.37^{ns}	2.84**	1.56^{ns}
CV (%)	33.51	35.86	12.21	9.11	27.84	27.86

ns: not significant by the Tukey test; FM: Fresh mass; MSD: Minimal significant difference; CV%: Coefficient of variation. Different letters indicate significant differeneces at p=0.05 (*) or p=0.01 (**).

ns: no significativo según el test de Tukey; MF: masa fresca; DMS: diferencia mínima significativa; CV%: Coeficiente de variación. Diferentes letras indican diferencias significativas para p=0,05 (*) o p=0,01 (**).

Conversely, cv. Hosui showed significant interactions between paclobutrazol (PBZ) doses and application forms, and between doses and evaluation dates. LSC averages were not affected by interactions between PBZ doses and application forms, or between doses and evaluation dates (table 1).

PBZ application affected plant growth throughout the evaluated time. Such an inhibitory effect decreased srpout length of cv. Santa Maria and Hosui plants. Similar results were reported for other fruit species such as mango (6, 22), citrus, apple (31), and cashew (21). Vegetative growth reductions after PBZ application are given by oxidation inhibition of *ent*-kaurene in the second stage of gibberellin biosynthesis (31).

Pear-tree sprout lengths showed exponential reductions after the interaction between PBZ application forms and doses (figure 1A, page 50). When compared to control treatments, plants receiving the highest leaf and soil doses (2.0 g PBZ linear meter canopy^{-1}) resulted 90 and 77% shorter than controls, respectively. However, plants receiving 1.5 g PBZ linear meter canopy^{-1} via leaf spray showed even greater sprout length reductions compared to higher doses. This greater efficacy of PBZ via foliar application may be attributed to direct contact with plant growing organs, where gibberellins are mostly synthesized.

Oliveira *et al.* (2012) evaluated the effect of foliar and soil PBZ applications and reported major growth reductions shortly after application of the highest foliar dose, which subsequently decreased, resuming normal growth. It is relevant mentioning that leaf applications require high doses to reduce plant growth.

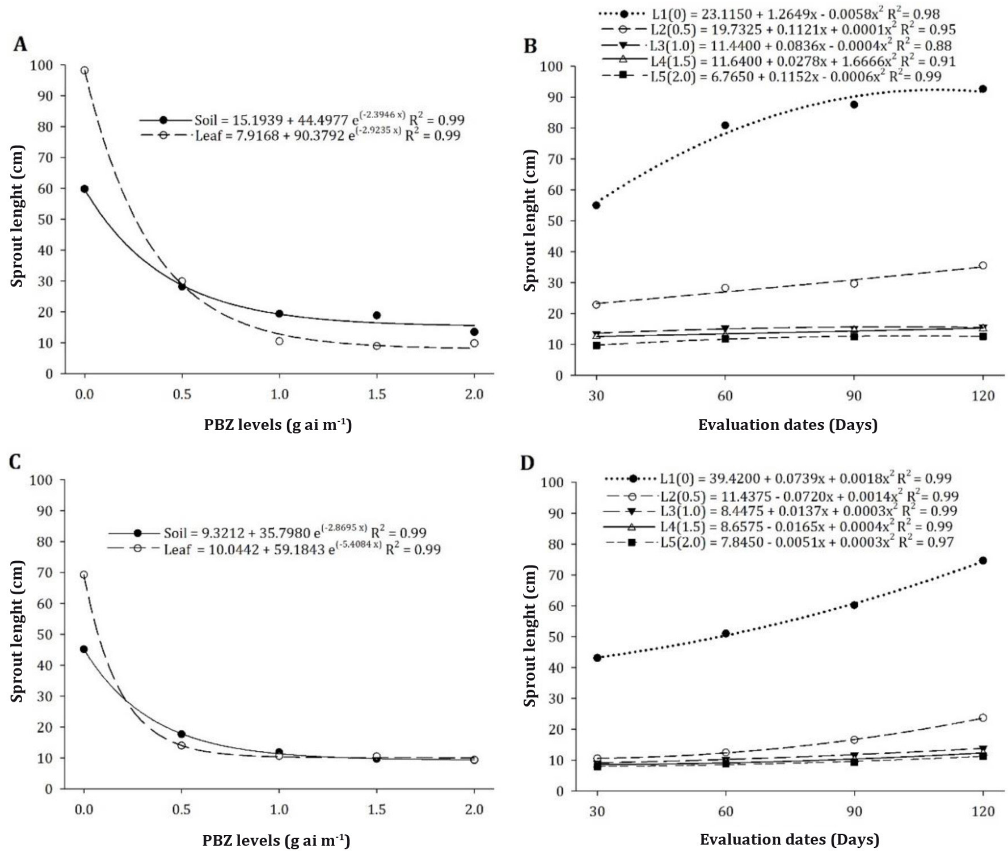

Figure 1. Sprout length of 'Santa Maria' (A and B) and 'Hosui' (C and D) pear cultivars as a function of PBZ levels and evaluation dates.

Figura 1. Longitud del brote de los cultivares de pera 'Santa María' (A y B) y 'Hosui' (C y D) en función de los niveles del PBZ y las fechas de evaluación.

PBZ action on sprout length (figure 1B) occurred shortly after application (at 30 days). Different doses reduced length of smaller sprouts, evidencing PBZ effectiveness in retarding pear-tree growth and development. Doses of 2.0 (86.44%), 1.5 (83.71%), and 1.0 (83.15%) g PBZ linear meter canopy^{-1} promoted the largest reductions in sprout length. Meanwhile, 0.5 g PBZ linear meter canopy^{-1} promoted the lowest reduction in sprout length compared to the control (61.65%) at the end of the evaluation period. Regardless of the dose, PBZ controlled sprout growth throughout the evaluation period, proving its efficiency and persistence in plants. Asín *et al.* (2007) evaluated the pear-tree cultivar 'Blanquilla' and observed that PBZ persistence increased residual effects.

Our results demonstrate that pear sprout length decreases as a function of PBZ and depends on application form and evaluation dates, proving to be efficient in maintaining tree vigor (figure 1C).

Sprout length reductions as a function of PBZ doses and evaluation dates, were fit to a quadratic model (figure 1D). From 30 days after, all PBZ-treated plants showed sprout growth reductions, while control plants continued growing. After 60 days, plants treated with 0.5 g PBZ linear meter canopy^{-1} showed a slight growth increase, evidencing the direct effect of PBZ on this variable. In this sense, Wongsrisakulkaew *et al*. (2017) also found significant reductions in sprout length of mangoes cv. Namdokmai-Sitong, two weeks after PBZ application.

For sprout diameter, Santa Maria plants showed a significant interaction between PBZ doses, evaluation dates and application form (table 1, page 49). Increasing PBZ doses reduced sprout diameter exponentially (figure 2A, page 51). In soil PBZ applications, sprout diameters progressively decreased as a function of PBZ doses.

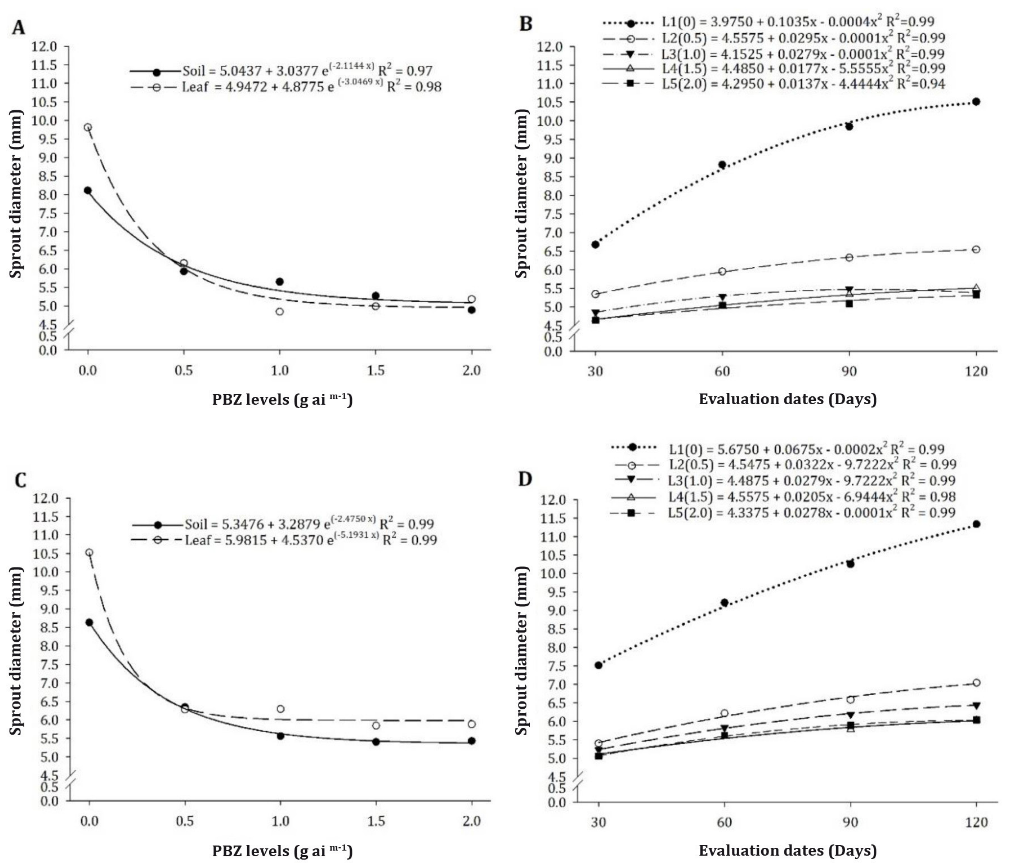

Figure 2. Sprout diameter of 'Santa Maria' (A and B) and 'Hosui' (C and D) pear cultivars as a function of PBZ levels and evaluation dates.

Figura 2. Diámetro del brote de los cultivares de pera 'Santa María' (A y B) y 'Hosui' (C y D) en función de los niveles del PBZ y las fechas de evaluación.

However, in foliar applications, the smallest reductions were verified at 1.0 and 1.5 g PBZ linear meter canopy^{-1}. At 2.0 g, plant sprout diameter decreased by 47.19 and 39.71% for foliar and soil applications, respectively. Sprout diameter reductions as a function of PBZ doses and evaluation dates, were fit to a quadratic model (figure 2B).

Over the evaluation period, sprout diameters of PBZ-treated plants were smaller than those of control plants. PBZ effect peaked from 30 days after, evidencing high efficiency on sprout diameter reduction. From 120 days after, sprout diameter of plants treated with 0.5 g PBZ linear meter canopy^{-1} increased greatly than the other treatments. Similar results were reported by Meena *et al.* (2014) for cashew trees and by Mir *et al.* (2015) for apricot trees.

'Hosui' plants showed the same trends as 'Santa Maria' plants for sprout diameter. Data on PBZ doses and application forms were best fitted to a quadratic model, while those on PBZ doses and evaluation dates were best fitted to an exponential model (figure 2C and 2D, respectively). Sprout diameter reductions with increasing PBZ doses were lower via soil (37.07%) than via leaf (44.11%) application modes.

After PBZ application, sprout diameters showed a downward trend as doses increased throughout the evaluation period. After 120 days, the largest reduction was observed at 2.0 g PBZ linear meter canopy^{-1} (46.69%), compared to non-treated plants. Similar results were reported by Sherif and Asaad (2014) for pear trees of the cultivar 'Le-Conte', and by Meena *et al.* (2014) for cashew trees. It is noteworthy that control plants had higher sprout diameters than the other treatments, all over the period.

Regardless of doses and application forms, PBZ-treated plants had reduced growth and a more compact aspect. Davenport (2007) pointed out that vegetative growth management is essential in fruit production since adjusting excessive sprouting can stimulate flowering and early fruiting in young plants.

In general, overall, the evaluation period, control plants exhibited larger sprout lengths and diameters than those of other treatments, including a continuous vegetative growth. Pear trees have vigorous growth, but vigor intensity varies with cultivars (29). When grown under intense rainfall, high-temperature, and in combination with frequent irrigation, pear trees show a more vigorous growth (15, 18). In this sense, Costa *et al*. (2004) highlighted that vegetative growth restrictions can increase fruiting by reducing growth competition between vegetative and reproductive parts. Specifically for pear trees, Kaur *et al.* (2020) evaluated the control potential of Pro-Ca and PBZ on vegetative growth, concluding that Pro-Ca was more effective, improving canopy light availability.

Total leaf soluble carbohydrate (LSC) levels varied as a function of the interaction among PBZ doses, PBZ application forms, and evaluation dates (figure 3). One hundred and twenty days after application of treatments, LSC contents in PBZ-treated plants were higher when soil applied (figure 3A). According to Lopes and Oliveira (2014), the ideal time for floral induction in pear trees under semi-arid tropical conditions is immediately before differentiation of vegetative *vs.* floral buds, after sprouting and accumulation of reserves. Prasad *et al.* (2014) observed that carbohydrate synthesis decreases close to floral induction, thus higher levels in this period can promote uniform flowering.

In figure 3B, lowercase letters compare application forms, capital letters compare PBZ doses in each application form. In figure 3C lowercase letters compare the evaluation dates and capital letters compare PBZ doses in each date. Means with the same letters do not differ by Tukey test ($p \leq 0.05$)

En la figura 3B, las letras minúsculas comparan las formas de aplicación, las letras mayúsculas comparan las dosis del PBZ en cada forma de aplicación. En la figura 3C, las letras minúsculas comparan las fechas de evaluación y las letras mayúsculas comparan las dosis del PBZ en cada fecha. Las medias con las mismas letras no difieren según la prueba de Tukey ($p \leq 0,05$)

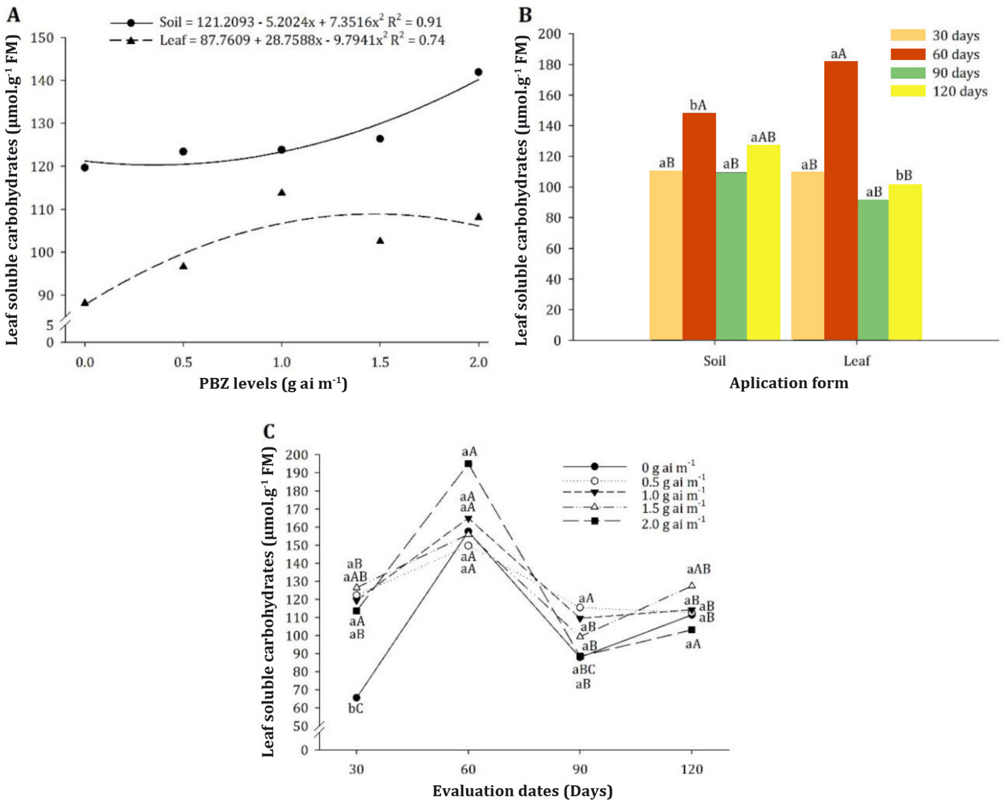

Figure 3. Leaf soluble carbohydrates (LSC) of 'Santa Maria' as a function of PBZ levels and application form at 120 days after PBZ (A); and LSC of 'Hosui' as a function of application form and evaluation dates (B); and LSC as a function of evaluation date and PBZ levels (C).

Figura 3. Carbohidratos solubles en hojas (LSC) de 'Santa María' en función de los niveles del PBZ y la forma de aplicación a los 120 días después del PBZ (A); y LSC de 'Hosui' en función de la forma de aplicación y las fechas de evaluación (B); y LSC en función de la fecha de evaluación y los niveles del PBZ (C).

The highest LSC concentrations in treated plants were 141.91 μmol.g^{-1} FM (2.0 g PBZ linear meter $canopy^{-1}$) and 113.77 μmol.g^{-1} FM (1.0 g PBZ linear meter $canopy^{-1}$) for soil and foliar applications, respectively. Plants with higher LSC amounts are expected to fulfill their demands at the beginning of floral induction. In studies on mango, Sherson *et al*. (2003) and Cavalcante *et al*. (2018) concluded that favorable flowering is induced by soluble carbohydrates availability, fundamental for bud development during dormancy and sprouting.

In cv. Hosui plants, LSC levels varied as a function of PBZ doses and application forms over evaluation time (figures 3B and 3C, respectively, page 52). In general, regardless of PBZ application form and dose, LSC accumulation was higher at 60 days after application and reduced at the end of the evaluation period (120 days after application). The largest increase was observed in plants receiving 1.5 g PBZ linear meter $canopy^{-1}$ (127.43 μmol.g^{-1} FM). Given that PBZ has anti-gibberellic action even at 120 days after application, and since total LSC storage is reduced, floral induction should be managed after this period.

Favoured floral bud formation due to LSC reductions has already been mentioned by Abdel Rahim *et al*. (2008) for mango and by Chen (1990) for litchi trees. However, sprouting was suppressed by PBZ in both application forms, and LSC contents decreased due to assimilates mobilization from source to sink tissues.

Concentrations of GA_3 in floral buds of 'Santa Maria' varied throughout the evaluation period for all treatments. At 30 days, foliar PBZ treatments (figure 4A, page 54) increased GA_3 contents compared to the control. Conversely, soil PBZ treatments (figure 4B, page 54) showed slight reductions in contents at 2.0 and 1.0 g PBZ per linear meter canopy, and increases at 0.5 and 1.5 g PBZ per linear meter canopy, alsowhen compared to the control. These results suggest that gibberellin-like substances were still active, thereby hindering PBZ inhibitory effect on plants with higher initial GA_3 concentrations. However, regardless of the application form, contents of GAs were strongly inhibited between 30 and 90 days after application. At this time, initial hormone (gibberellin-like substances) levels were high, suggesting that PBZ effectiveness may be related to target substance availability. GA biosynthesis is blocked by PBZ, but plant responses vary with climate, application time and form, plant age and vigor, or dose (9).

Regardless of the application route, PBZ was effective in reducing pear-tree sprouting during the evaluation period, inhibiting GA biosynthesis efficiently. In both application routes, GA_3 concentrations reduced significantly at 0.5, 1.5, and 2.0 g PBZ per linear meter canopy, compared to the control. These reductions occurred between 120 and 150 days after application, when floral induction in pear trees occurs in semi-arid conditions.

A decline in shoot growth preceding floral induction is related to low GAs levels, promoting the transition from vegetative to reproductive meristem (15). PBZ-induced reduction in GA levels also mobilizes essential carbohydrates for floral induction, developing flower buds and produce floral initiation. Thus, when mediated by PBZ, a florigenic promoter (PF) is up-regulated, while a vegetative promoter (PV), characterized by high endogenous GAs concentrations, is down-regulated (9).

According to Burondkar *et al.* (2016), not all GAs play the same role in inducing flower buds. Declines in GA_1 are important for bud formation and floral induction, while declines in GA_3, GA_4, and GA_7 act on flower bud initiation.

Concentrations of GA_3 in flower buds of 'Hosui' varied across the evaluation period for all treatments. At 60 days, both application forms had increased GA_3 levels compared to the control. Foliar PBZ (figure 4C, page 54) markedly reduced GA levels along the evaluation period, except for the doses of 1.5 and 2.0 g PBZ per linear meter canopy. In this treatment, GA levels tended to surpass control after 120 days. This result can be attributed to direct contact of PBZ with leaf surfaces and/or plant growth points, where endogenous GAs are synthesized, thus reducing their activity through the oxidation of *ent*-kaurene to *ent*-kaurenoic acid.

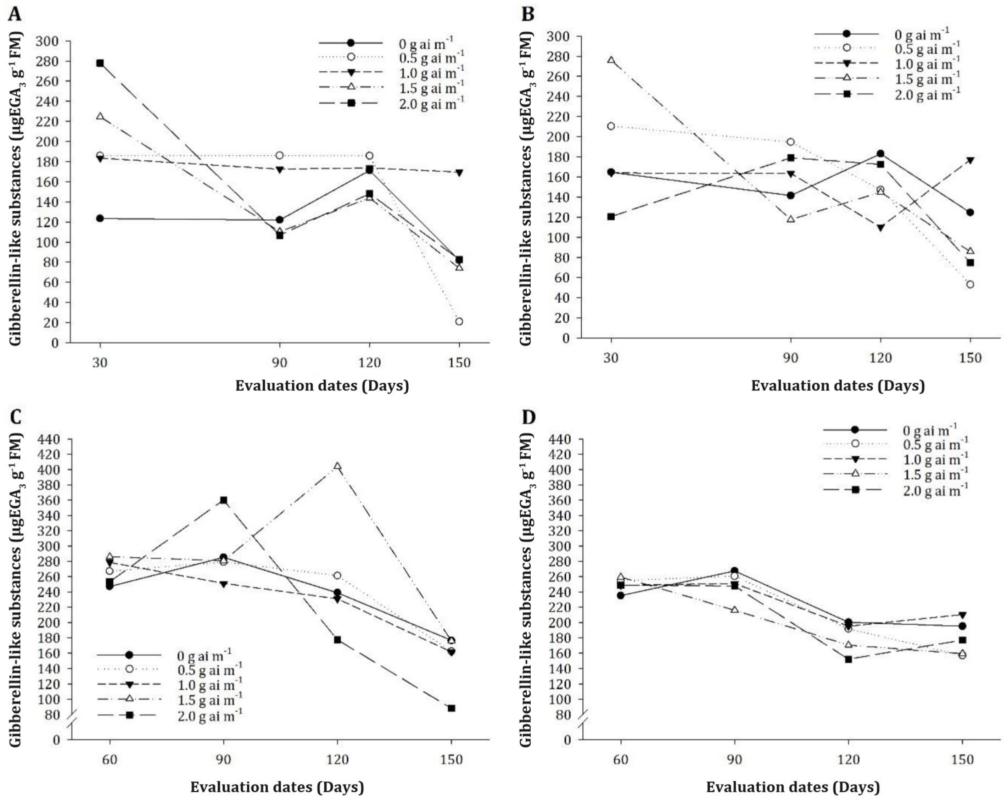

Figure 4. Gibberellin-like substances concentrations ($\mu gEGA_3\ g^{-1}$ FM) in buds of 'Santa Maria' (A and B) and 'Hosui' (C and D) pear cultivars as a function of PBZ levels and forms of application (A and C - leaf; B and D - soil) after PBZ application.

Figura 4. Concentraciones de sustancias similares a la giberelina ($\mu gEGA_3\ g^{-1}$ FM) en brotes de cultivares de pera 'Santa Maria' (A y B) y 'Hosui' (C y D) en función de los niveles del PBZ y las formas de aplicación (A y C - hoja; B y D - suelo) después de la aplicación del PBZ.

After the first evaluation, plants treated via soil (figure 4D) with the doses of 0.5 and 1.0 g PBZ per linear meter canopy reduced GA levels gradually. Meanwhile, those treated with the doses of 1.5 and 2.0 g PBZ per linear meter canopy showed a sharp increase followed by a drastic reduction in the last evaluations (at 120 and 150 days). The latter may be due to PBZ action mode and GA type in plant tissue, as different GA types act in different ways. Fagan *et al.* (2015) reported that GA biosynthesis is regulated by feedback control, in which GAs control their synthesis influenced by environmental factors (*e.g.*, photoperiod and temperature). After 120 days, until the last evaluation date, GAs concentrations were kept at low levels in comparison to the beginning of the experiments, making plants suitable for floral induction, and confirming soil PBZ efficiency.

As mentioned, PBZ regulates vegetative growth by blocking P450 monooxygenase enzymes and preventing oxidation of *ent*-kaurene to *ent*-kaurenoic acid (31). Therefore, the lower GA concentrations measured over the experiments proves that PBZ effectively reduces GAs activity in sprout growth. Floral induction is expected to occur from 120 to 150 days after PBZ application. In this sense, according to Huang *et al*. (1986), floral induction is given by changes in hormones (Auxins, Cytokinins, Gibberellins), due to GA reductions, promoting the transition from vegetative to reproductive meristem, which is when floral induction should be managed.

Conclusions

Both soil and foliar paclobutrazol applications inhibit gibberellin biosynthesis in 'Santa Maria' and 'Hosui' pear-tree cultivars. Paclobutrazol application also affects total soluble carbohydrate contents in leaves and reduces sprout growth in both cultivars, especially at 0.5, 1.5, and 2.0 g PBZ per linear meter canopy after 120 days of application. Although paclobutrazol can be potentially used in pear-tree management, further studies are still required to determine specific practices in tropical semi-arid regions.

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Leaf area and its impact in yield and quality of greenhouse tomato (*Solanum lycopersicum* L.)

El área foliar y su impacto en el rendimiento y calidad de tomate en invernadero (*Solanum lycopersicum* L.)

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Abstract

This study aimed to estimate the tomato leaf area index (LAI) by comparing two methods (destructive and interception of photosynthetically active radiation) and the consequent relationship to fruit yield and quality. The experiment was carried out in a greenhouse with tezontle (red volcanic scoria) as the substrate and a drip irrigation system. The experiment consisted of three treatments: T1, T2 and T3, with one, two and three stems per plant, respectively. The LAI was measured with a ceptometer that estimates the intercepted radiation above and below the canopy. Maximum LAI was found at 1413 cumulative growing degree days (CGDD). Those indexes were 3.69, 5.27 and 6.16 for T1, T2 and T3, respectively. Individual correlation models were fitted linearly between the two methods. The R^2 values were 0.98, 0.99 and 0.99 with yields of 20, 18 and 17 kg m^{-2} for T1, T2 and T3, respectively. In addition, T1 produced better fruit size quality with approximately 69, 23 and 8% classified as first, second and third class, respectively. Only 1% was classified as a small fruit. Increasing the number of stems per plant increased the LAI and fruit number but decreased fruit size.

Keywords
Solanum lycopersicum L. • ceptometer • photosynthetically active radiation

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Resumen

El objetivo del estudio fue estimar el índice de área foliar (LAI) del tomate comparando dos métodos (destructivo e intercepción de la radiación fotosintéticamente activa) y su relación con el rendimiento y calidad de frutos. El experimento se realizó en invernadero usando como sustrato tezontle bajo riego por goteo. El experimento consistió en tres tratamientos (T) de uno (T1), dos (T2) y tres (T3) tallos por planta. Para estimar el IAF se utilizó un ceptómetro, el cual estima la radiación interceptada por encima y por debajo del dosel vegetal. El máximo LAI se presentó a los 1413 Grados-Día Desarrollo acumulados, los cuales fueron 3.69, 5.27 y 6.16 para cada tratamiento respectivamente. Se obtuvo coeficiente de correlación lineal (R^2) entre ambos métodos de 0.98, 0.99 y 0.99 con un rendimiento de 20, 18 y 17 kg m^{-2} para T1, T2 y T3. El T1 produjo mejor calidad en tamaño con 69, 23, 8 y 1% frutos de primera, segunda, tercera y pequeños. También se encontró que el LAI y el número de frutos incrementaron al aumentar el número de tallos por planta. Sin embargo, el tamaño del fruto disminuyó.

Palabras claves

Solanum lycopersicum L. • ceptómetro • radiación fotosintéticamente activa

Introduction

Tomato (*Solanum lycopersicum* L.) is the most common greenhouse-grown vegetable, greatly demanded worldwide. The intensive tomato greenhouse production system employs indeterminate variety types with low planting densities ranging from two to three plants per square meter. Side shoots are often pruned, leaving a single stem that reaches more than seven meters long, achieving 15 or more bunches per plant in a single season per year at harvest (3). This production system is relatively new in Mexico and has contributed to the increase in cultivated area, productivity, profitability and quality (20, 21).

Plant density and other management practices, such as pruning, side shoot removal and plant staking, are considered important in intensive production systems (6, 24). Pruning reduces plant height and the crop-growing period, and induces the plant to develop better quality fruit in terms of size and yield (5, 18, 29, 36, 37). Side shoot removal reduces internal competition for water, nutrients and assimilates, and the string trellis method supports the plant and improves canopy illumination (24). However, the manipulation of plant density optimizes the intercepted radiation (28, 37). A high plant density increases the number of small fruit (20).

The leaf area index (LAI) is an important variable for studying crop growth and development. In addition, it is the basis for estimating crop water and nutrient requirements, bioenergy efficiency, and potential crop damage. There is a close relationship between LAI and solar radiation interception, which is associated with photosynthesis and transpiration processes, both of which are strongly linked to biomass accumulation and productivity. Therefore, LAI is a variable used to quantify crop growth and yield (14, 17). The electromagnetic spectrum region of greatest interest in agriculture is photosynthetically active radiation (PAR), which has wavelengths between 400-700 nm. It is important to know the spatial and temporal distribution of PAR interception by crops, as it is the basis for the analysis of biological processes (13).

Direct LAI determinations are often destructive and require instruments to penetrate the leaf surface (40). As a variant of this method, Rodríguez (2000) used digital photographs and image interpretation techniques to measure leaf area and determine LAI in potato crops. However, there are indirect, non-destructive methods that allow for a quick in-field determination of the relationship between radiation penetration and canopy structure.

To measure PAR, devices such as the ceptometer have often been used. This device measures photosynthetic photon flux density (PPFD), defined as the amount of photosynthetically active photons falling on a given surface per unit area per unit of time, and is expressed in µmol m^{-2} s^{-1}. De La Casa *et al.* (2008) obtained a correlation of R^2 = 0.86 when comparing the LAI measured by the ceptometer with measurements obtained from digital photographs of potato (*Solanum tuberosum* L.) crops. Mendoza-Pérez *et al.* (2017)

obtained promising results when comparing the LAI values produced by the ceptometer as compared to direct measurements obtained using an LI-3100C leaf integrator on Poblano pepper (*Capsicum annuum* L.).

The development of many plant species and organisms is mainly controlled by temperature (thermal time). The basic concept of thermal time is that many phenological and growth crop processes proceed in direct relation to the accumulated temperature experienced by the crop. Below a base temperature, no thermal time accumulates and crop development ceases. Above an optimum temperature, crop development is not enhanced; presumably, between these temperature thresholds, the plant must accumulate a certain amount of thermal time to complete development (22, 32).

There is a clear need to identify the growth and development stages of tomato crops in terms of irrigation and fertilization planning in a controlled environment. LAI, as influenced by the number of stems per plant, is one of the agronomic management variables associated with productivity. The greater the number of stems per plant, the greater the leaf area. However, the overall production (fruit quantity and quality) can be affected, as reported by Mendoza-Pérez *et al*. (2017). Therefore, this study aimed to estimate the LAI of tomato with an indirect method (ceptometer), compare it with a direct method, and analyze the relationship between LAI and the biomass, yield and quality of fruit grown under greenhouse conditions.

Materials and methods

Description of the experiment

The experiment was carried out in a greenhouse located on the Graduate College, Montecillo Campus, (19°28’05” North latitude and 98°54’31” West longitude, at 2244 m a. s. l.). Saladette-type tomato variety ‘Cid’ was planted. Seeding occurred on March 5th, transplanting on April 20th and harvesting finished on September 20th, 2015. Plantation frame was 40 cm x 40 cm. Seedlings were transplanted in 35 × 35 cm black polyethylene bags using tezontle (red volcanic scoria) as the substrate.

Treatments consisted of three management conditions as a function of stems per plant: T1 = one stem, T2 = two stems and T3 = three stems per plant. The main stem in T1 was left to grow. T2 and T3 maintained the main stem with one and two secondary stems, respectively. The area of each treatment was 53 m^2. Each main treatment was established in plots with two beds 20 m long and 1.35 m apart, resulting in a crop density of 3 plant m^{-2}. A split-plot randomized complete block experimental design was used, with four replicates.

The drip irrigation system employed on-surface driplines, 16 mm in diameter with self-compensating drippers of 4 L h^{-1} at 40 cm apart and an operating pressure of 0.7 kg cm^{-2}. Irrigation with Steiner’s nutrient solution was applied throughout the season. The flow rate (Q) was 0.155 L per plant for the first 30 days after transplant (DAT), which corresponded to the initial stage, while 0.462 L and 0.891 L per plant were applied in vegetative and reproductive stages, respectively. At maximum demand (production), the flow rate increased to 1.650 L per plant.

Estimate of LAI using a ceptometer

The LAI measurement was performed with an AccuPAR LP-80 ceptometer (PAR/LAI Ceptometer, Decagon Devices, Pullman, Washington, USA), which estimates PAR under field conditions and consists of a 1-m long bar with 80 sensors divided into eight segments. Readings were taken every 8 days starting at 20 days after transplant (date on which the division of the number of stems began) until the harvest of the tenth cluster of fruit (September 20th, 2015). Measurements were made above and below the canopy, with six replications per treatment. The intercepted PAR values were estimated from two positional levels of radiation, one above the foliage to obtain the incident PAR (PARa) and the other below the foliage to obtain the PAR reaching the ground (PARd) in μmol m^{-2} s^{-1} (Equation 1).

$$PAR = \frac{PARd}{PARa} \qquad (1)$$

Once the PAR was obtained, it was combined with other variables, such as leaf angle factor, solar zenith angle and radiation extinction coefficient. Finally, an adjustment was made to estimate the LAI of the plant according to Equation 2 proposed by Campbell and Norman (1989).

$$LAI = \frac{\left[\left(1-\frac{1}{2K}\right)\ Fb-1\right] ln\left(\frac{\mathrm{PARd}}{\mathrm{PARa}}\right)}{A\ (1-0.47\ Fb)} \tag{2}$$

where:

K = the radiation extinction coefficient that considers a spherical angular distribution parameter k = 1

Fb = the fraction of direct radiation with respect to global solar radiation (0.25)

A = the absorption coefficient of the canopy that is equal to 0.86 (Campbell and Norman, 1989).

Ceptometer readings were taken only under clear sky conditions during the period of maximum solar insolation (noon). Thus, the zenith angle was as low as possible, and factor Fb corresponded to high fractions of direct solar radiation that made it possible to homogenize data. LAI measurements were made with the destructive method (extracting the plant, separating the leaves and measuring the leaf area) with the electronic leaf area integrator (Area Meter Model LI-3100, Decagon Device, Inc. Lincoln, Nebraska, USA) to compare that data with that obtained from the ceptometer. In addition, plant LAI was calculated using Equation 3, as described by Reis *et al*. (2013).

$$LAI = \frac{FA\ X\ NP}{TA} \tag{3}$$

where:

LAI = leaf area index ($m^2\ m^{-2}$)

FA = the average foliar area of three plants (m^2)

NP = the number of plants per m^2 and TA is the total area considered to be 1 m^2

Description of variables

Growing degree days (GDD) for each day were calculated using Equation 4, as proposed by Ojeda-Bustamante *et al*. (2004).

$$\begin{aligned} GDD &= T_a - T_{c\text{-}min},\ \text{if } T_a < T_{c\text{-}max} \\ GDD &= T_{c\text{-}max} - T_{c\text{-}min},\ \text{if } T_a \geq T_{c\text{-}max} \\ GDD &= 0,\ \text{if } T_a \leq T_{c\text{-}min} \end{aligned} \tag{4}$$

where:

T_a = daily air temperature

$T_{c\text{-}max}$ and $T_{c\text{-}min}$ = maximum and minimum air temperature. The temperature range for tomato growth is 6 and 29°C (Iglesias, 2015).

The cumulative GDD values for n days are expressed as follows:

$$CGDD = \sum_{i=1}^{n} GDDi \tag{5}$$

where:

i = the number of days elapsed from an initial day of interest, usually the transplant date or the first day of a stage

GDD = growing degree day for each day

For dry weight determination, plant stems, roots, leaves and fruit were weighed fresh and then placed on a forced air oven at 70°C for 72 hours for complete dehydration. Finally, all samples were weighed using a digital scale 5000p model with 0.001 g resolution.

To estimate yield, eight plants were selected per treatment. Plant height and the number and size of fruit were also evaluated. Classification of fruit sizes was made with the following categories (large, medium, small and tiny) based on the equatorial diameter using the fruit diameter Mexican standard NMX-FF-009-1982.

Statistical analyses

Mean differences among treatments were separated using Tukey's test at P ≤ 0.05 (MINITAB® Release 14 Statistical Software). Correlation models were fitted to each experimental unit and tested in terms of significance level (α = 0.05) and R^2 values.

Results and discussion

Photosynthetically active radiation (PAR)

Photosynthetically active radiation intercepted in the canopy at the initial stage was approximately 900 μ mol m^{-2} s^{-1} in all treatments. Plant leaf area and, consequently, biomass increased over time. The maximum amount of radiation intercepted was 1300 μ mol m^{-2} s^{-1}, which coincided with the maximum LAI at flowering (seventh floral cluster and 1431 CGDD). Afterwards, it started to decrease (from June through September), coinciding with the rainy months in the study area (figure 1). Mendoza-Pérez *et al.* (2017) obtained similar PAR trends in greenhouse-cultivated Poblano pepper. In addition, García-Enciso *et al.* (2014) obtained similar PAR trends in the production and quality of greenhouse-grown tomato fruit.

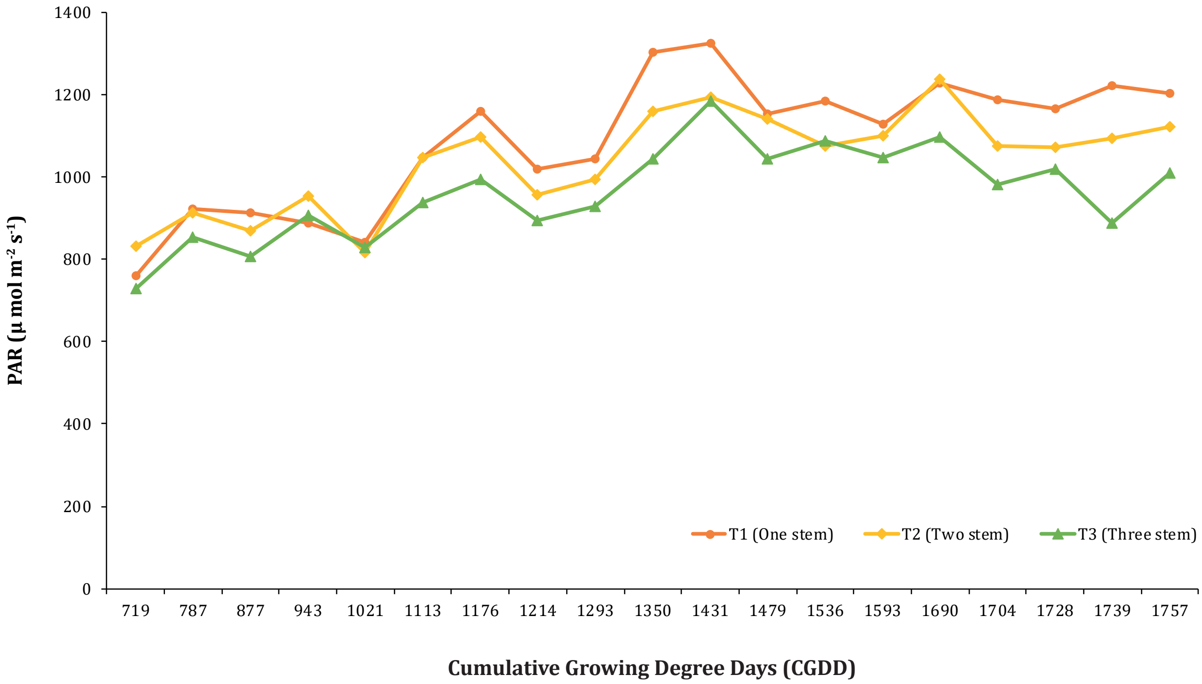


Figure 1. Photosynthetically active radiation intercepted in the canopy throughout the growing season.

Figura 1. Radiación fotosintéticamente activa interceptada en el dosel de la planta durante todo el ciclo de desarrollo del cultivo.

Leaf area index (LAI)

T3 had the highest LAI, with the highest value of 6.16 at flowering (1431 CGDD), after which it decreased. At 1113 CGDD, LAI differed among treatments due to differences in the number of stems. Figure 2 shows the LAI values obtained with the ceptometer and the destructive method. The highest value of LAI was found at 1431 CGDD with 3.69, 5.27 and 6.16 $m^2.m^{-2}$ for T1, T2 and T3, respectively.

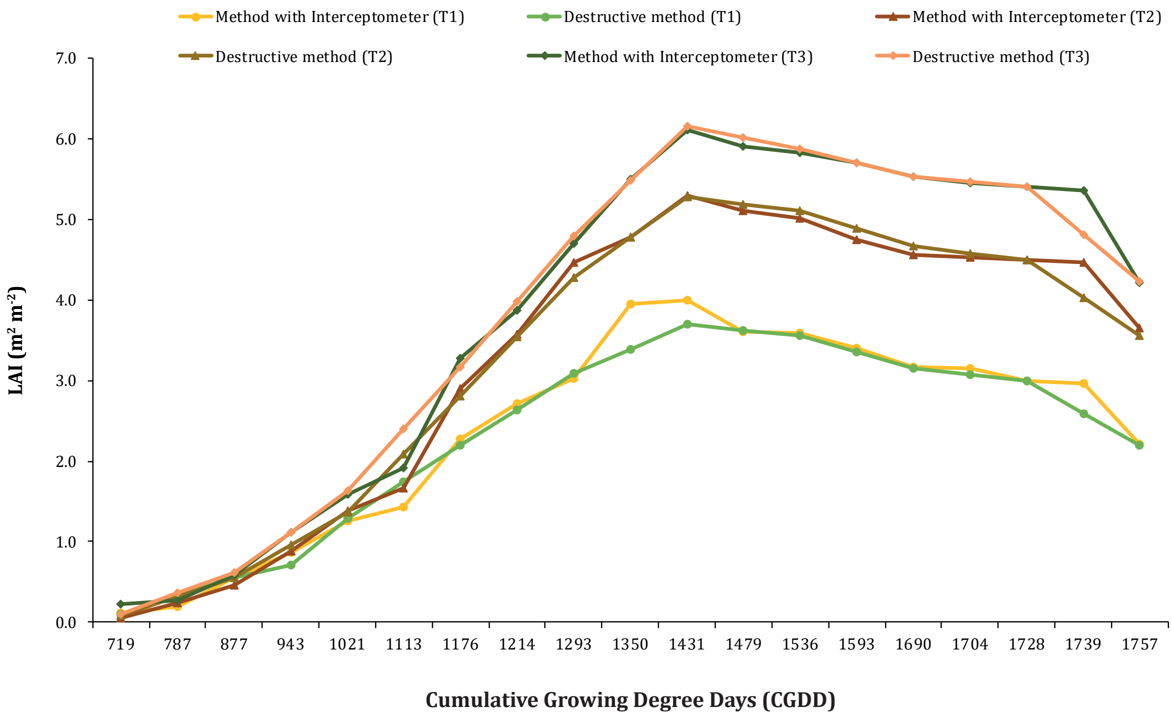


Figure 2. Measured (destructive method) and estimated (ceptometer) LAI for all treatments.
Figura 2. IAF medido (método destructivo) y estimado (ceptómetro) para todos los tratamientos.

LAI started to decrease once maximum growth and development were achieved due to leaf senescence. These results agreed with those reported in a tomato trial by Vargas (2012), who found a LAI of 4.0 $m^2.m^{-2}$ for a mixture of tezontle and sawdust (20:80 ratio), a LAI of 5.2 m^2 m^{-2} (30:70 ratio) with the same mixture and an LAI of 6.2 m^2 m^{-2} using tezontle only.

Mendoza-Pérez *et al.* (2017) obtained similar results in the cultivation of Poblano pepper as a function of the number of stems per plant grown under greenhouse conditions. Rojas (2015) evaluated the impact on tomato development and yield at different percentages of PAR block during winter, obtaining a LAI of 3000, 2100, 2100, 2000 and 1750 cm^2 for blocks of 50, 35, 60, 25 and 75%, respectively.

Coefficient of determination

The data showed a linear relationship in both methods (measured and estimated), with similar slopes (angular coefficient). The R^2 values were 0.98, 0.99 and 0.99 for T1, T2 and T3, respectively ($P < 0.01$). The dispersion of LAI data explained by direct determination was the result of the effects of the measurements of a 1 m^2 area as compared with measurements in more replicates obtained with the ceptometer (figure 3, page 63). These data were similar to those reported by De la Casa *et al.* (2012) in potato, achieving R^2 values of 0.80, and to those of Mendoza-Pérez *et al.* (2017) in Poblano pepper, attaining R^2 values of 0.82 for treatments with two stems, 0.94 for treatments with three stems and 0.99 without pruning.

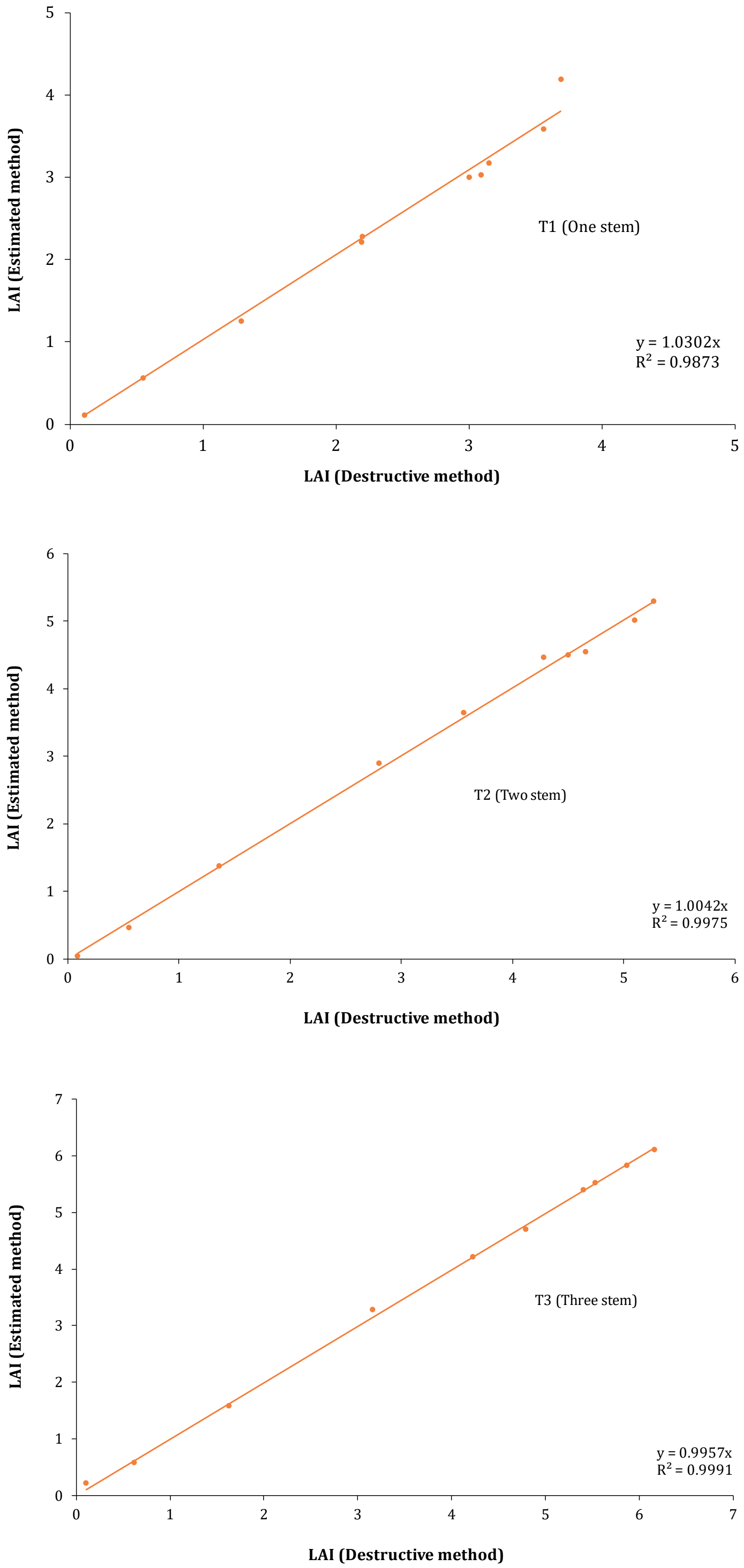


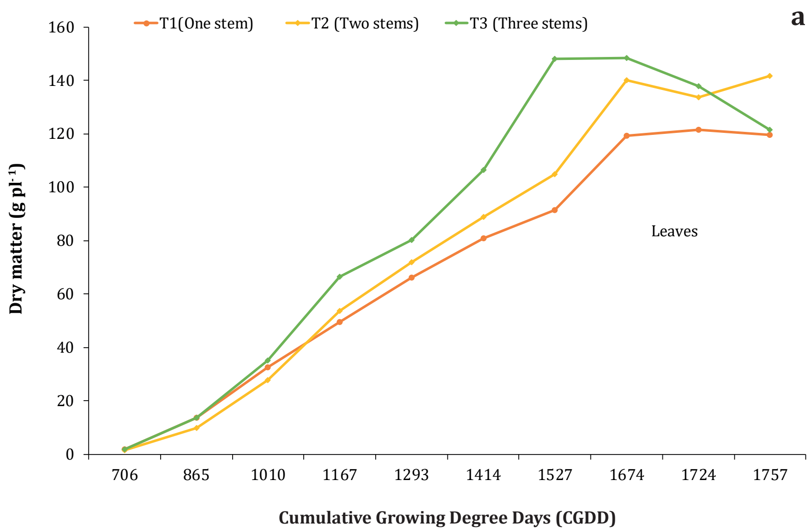
Figure 3. Linear regression coefficient of leaf area index for both methods.
Figura 3. Coeficiente de regresión lineal del índice de área foliar para ambos métodos.

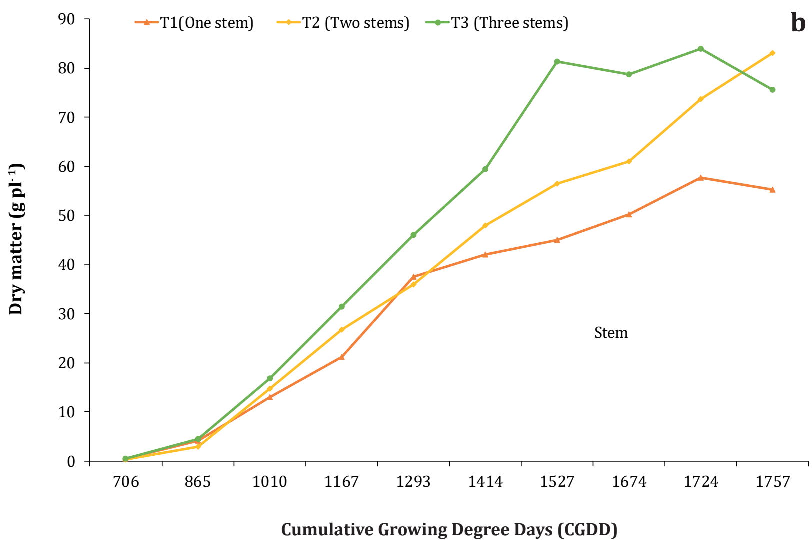
Dry matter

The distribution of dry matter among different plant organs plays a fundamental role in crop production, since growth is achieved by the capacity of the plant to accumulate biomass in the organs (20). Figure 4 shows the partition of dry matter in different plant organs (leaf, stem, fruit, root and flower), attained at 1167 CGDD. These results are similar to those reported by Betancourt and Pierre (2013), Hernández *et al*. (2009) and Gandica and Peña (2015). Therefore, there was an increasing accumulation of dry matter in the aerial part where the fruit contributed much of this content in the full production phase, as shown by Gandica and Peña (2015). This variable had a direct relationship with the LAI (the higher the biomass, the higher the LAI). However, both fruit yield and quality were affected because of processes within the plant. In the same way, Villegas *et al*. (2004) found similar trends in tomato under greenhouse conditions (12).

Plant height

Plant height was low from transplant to 658 CGDD. Between 743 and 819C CGDD, it increased markedly until reaching 275, 280 and 305 cm for T1, T2 and T3, respectively (figure 5, page 66). The shoot apex of the plant was cut once the 10 clusters were accumulated.





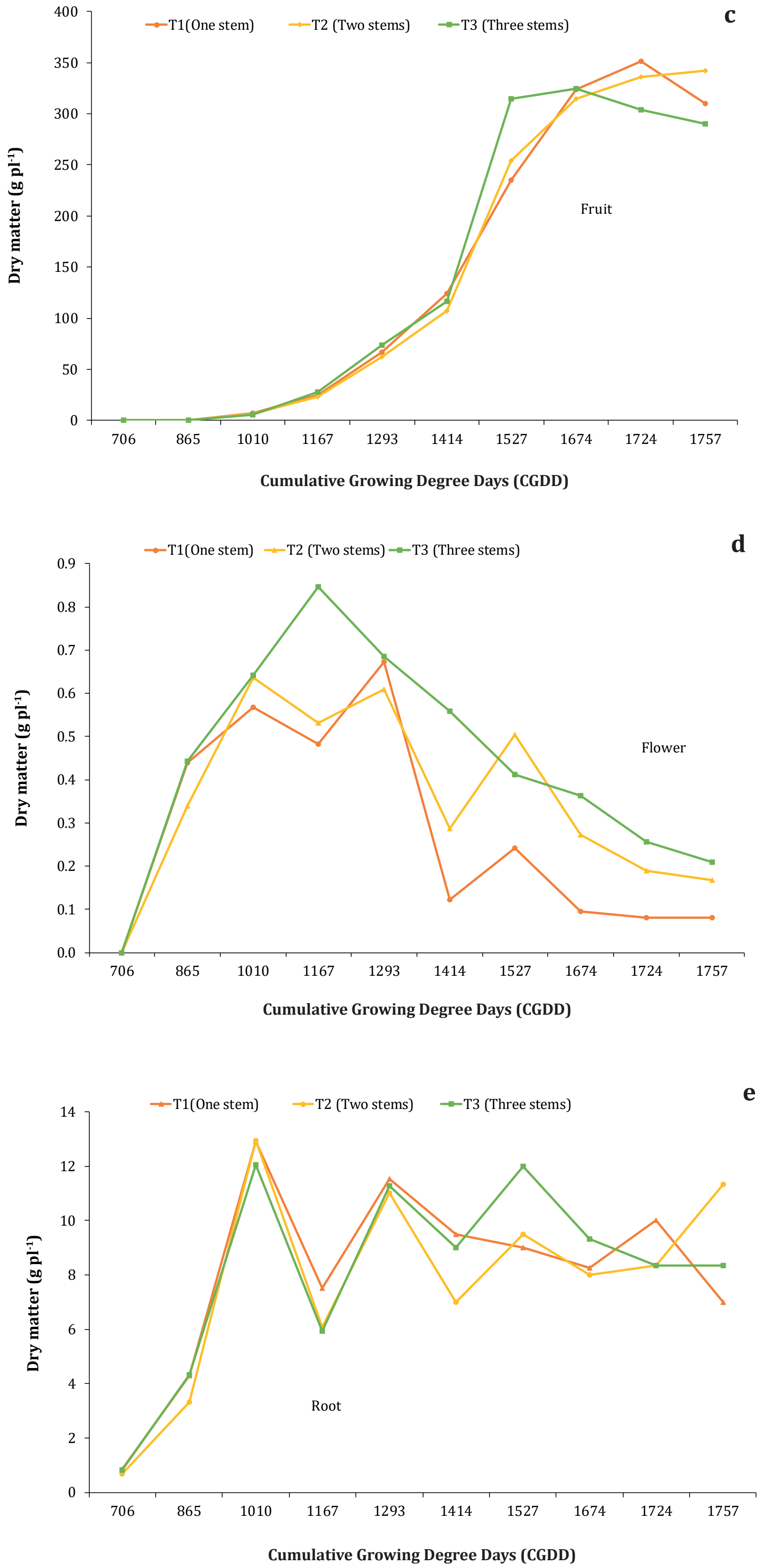


Figure 4. Dry matter accumulated in leaves (a), stems (b), fruit (c), flower (d) and root (e).
Figura 4. Materia seca acumulada en hojas (a), tallos (b), frutos (c), flor (d) y raíz (e).

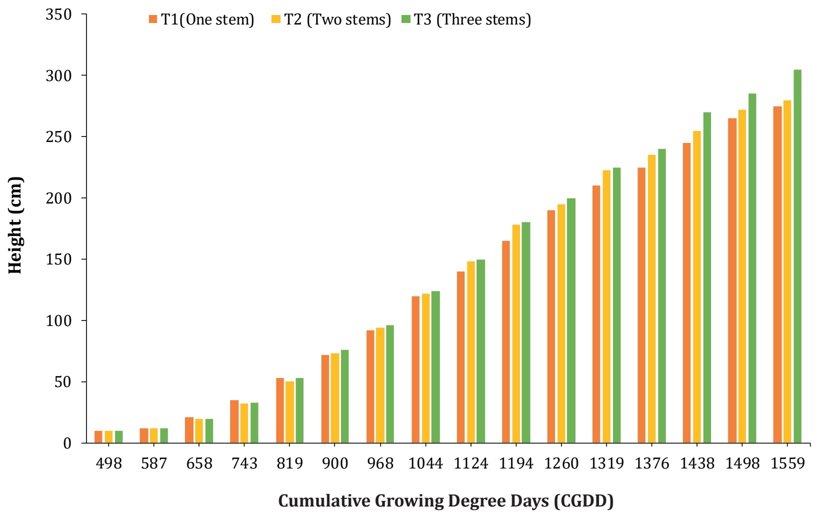


Figure 5. Plant height in all treatments.

Figura 5. Altura de planta en todos los tratamientos.

Growth dynamics followed a sigmoid-like curve. The curve suggested exponential growth from 743 CGDD in all treatments. At 900 CGDD, the T3 growth rate accelerated, resulting in more stems and leaves that were responsible for intercepting light for photosynthesis. This performance was similar to that reported by Núñez-Ramírez *et al*. (2012).

Total fruit number per plant

Table 1 shows the total fruit number obtained in each treatment. The values were 186, 234 and 247 fruit per m^{-2} for T1, T2 and T3, respectively. Núñez-Ramírez *et al*. (2017) reported similar results for fruit number, quality and final yield as influenced by nitrogen fertilization. The values found in this work show that T2 and T3 had plants with a higher fruit number compared to T1.

Tabla 1. Número total y rendimiento de frutos en cada tratamiento.

Table 1. Total fruit number and yield of fruit in each treatment.

Treatments	Fruit number	Yield (kg m^{-2})
T1(One stem)	186 **b**	20 **a**
T2 (Two stems)	233 **a**	18 **ab**
T3 (Three stems)	247 **a**	16 **b**
CV (%)	**32.2**	**2.0**

CV = coefficient of variation (%).

CV = Coeficiente de variación (%).

Yield per plant

The final yield obtained was 20, 18 and 16 kg m^{-2} for T1, T2 and T3, respectively. These data coincide with those reported by Corella *et al*. (2013), who obtained yields of 23.43 and 18.55 kg m^{-2} for the same crop under similar conditions. Finally, T3 had the lowest yield (16 kg m^{-2}). However, no literature was found that considered the yield and quality of three-stem tomato plants. Furthermore, De la Rosa-Rodríguez *et al*. (2016) obtained similar results when comparing tomato production and quality in open and closed hydroponic systems. They achieved yields of 17.5 and 16.9 kg m^{-2} with a density of 4 plants m^{-2}.

Fruit size classification

Figure 6 (page 67) shows that the number of stems per plant can strongly affect the final fruit size. T1 had approximately 69, 23, 8 and 1% of the fruit classified as large, medium, small and tiny, respectively.

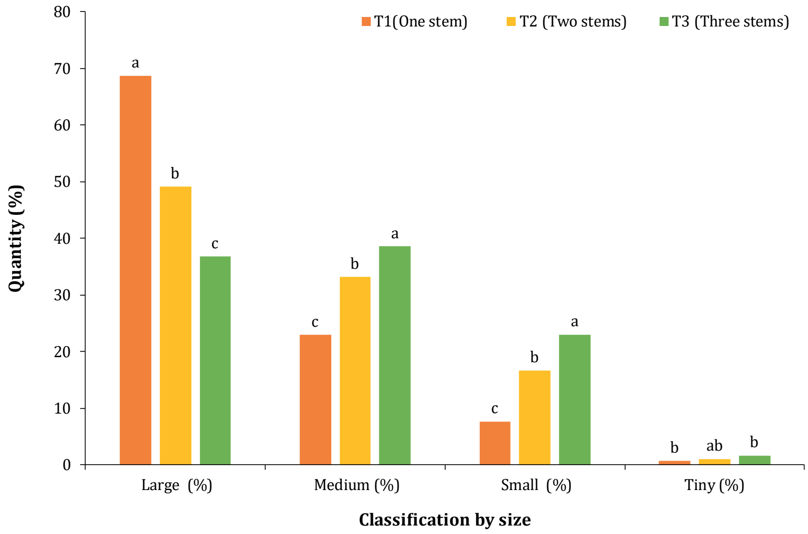


Figure 6. Classification of tomato fruit size quality.

Figura 6. Clasificación de calidad en tamaño de los frutos de tomate.

These results were similar to those reported by Rodríguez *et al*. (2008), who found that 60, 20, 10 and 10% corresponded to extra size, first and second class as well as loss, respectively.

For T2, approximately 49, 33, 17 and 1% were classified as large, medium, small and tiny, respectively. These results were similar to those reported by Quintana-Baquero *et al*. (2010), who found that 9, 52, 27, 11 and 1% corresponded to extra size, first, second, third and fourth classes, respectively. Finally, in T3, the percentages of large, medium, small and tiny were 37, 39, 23 and 1%, respectively. However, T3 produced more medium-sized and small fruit compared to T1 and T2, respectively.

Conclusions

Estimation of the LAI of tomato using a ceptometer proved to be a fast, useful and statistically reliable method. LAI was strongly correlated with yield by increasing the fruit number per plant. It also increased the amount of photosynthetically active radiation intercepted by the canopy, which favored photosynthetic efficiency per unit area. This process can be mainly attributed to a higher concentration of chlorophyll per unit leaf area.

The number of stems per plant was shown to increase the LAI, dry matter accumulation, plant height and fruit number. However, fruit size (quality) decreased. Further studies with a higher nutrient concentration should be conducted, since it could be the key to maximizing the yield potential of two-stem plants.

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Effect of forage aqueous extracts on *Glycine max* L. Merr., *Zea mays* L. and *Bidens pilosa* L.

Efecto de extractos acuosos de forraje sobre *Glycine max* L. Merr., *Zea mays* L. y *Bidens pilosa* L.

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Abstract

The aim of the present study was to investigate the potential allelopathic effects of isolated or intercropped aqueous forage extracts on the physiological performance of soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) seeds, as well as their phytotoxicity to the weed species *Bidens pilosa* L. Aqueous extracts were prepared at a concentration of 5%, and the tests with soy and corn were conducted by wetting the germination papers with different treatments and performing procedures within the standards of the Brazilian Rules of Seed Analysis. Tests with the cover plant extracts on the seeds of *B. pilosa* used BOD-type repetitions. Fifty weed seeds were placed in a gerbox with filter paper moistened with the different treatments with five repetitions. Distilled water was used as the control in all tests. Extracts of *Avena strigosa* Schreb and *Raphanus sativus* L. (radish), cultivated individually and in combination, increased the seed germination percentage (%G) and the length of soybean seedlings. Similarly, extracts of *Pennisetum glaucum* (L.) R.Br and *Crotalaria spectabilis* Roth, in individual and combined cultivation, provided gains in corn %G. Furthermore, all tested extracts suppressed germination and decreased the speed of the germination index of the hairy beggarticks when compared to the control, with the radish extract showing the greater reduction effect on the %G of the weed plant *B. pilosa.*

Keywords
allelopathy • stimulating effect • weed suppression

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Resumen

El objetivo del presente estudio fue investigar la posible influencia de la alelopatía de los extractos acuosos de cultivos forrajeros, aislados o en consorcio, sobre rendimiento fisiológico de las semillas de soja [*Glycine max* (L.) Merr.] y de maíz (*Zea mays* L.), además de su fitotoxicidad en la hierba *Bidens pilosa* L. Se prepararon extractos acuosos a una concentración del 5%. Los estudios con soja y maíz se llevaron a cabo humedeciendo los papeles de germinación correspondientes a los diferentes tratamientos y se realizaron dentro de los estándares de las Reglas de Análisis de Semillas de Brasil. Estudios con los extractos en las semillas de *B. pilosa* se llevaron a cabo en una cámara de tipo B.O.D. Se colocaron cincuenta semillas de malezas en caja gerbox conteniendo papel de filtro humedecido con los diferentes tratamientos en cinco repeticiones. El agua destilada se utilizó como control en todas las pruebas. Los extractos de *Avena strigosa* y *Raphanus sativus* L., cultivados tanto en forma aislada como en consorcio, aumentaron el porcentaje de germinación de semillas (%G) y la longitud de las plántulas de soja. Asimismo, los extractos de *Pennisetum glaucum* (L.) R.Br. y *Crotalaria spectabilis* Roth, cultivados aislados y en consorcio, proporcionaron ganancias en el %G de maíz. Además, todos los extractos evaluados suprimieron la germinación y redujeron la velocidad del índice de germinación de la maleza en comparación con el control, siendo el extracto de *R. sativus* el que más redujo el % G de *B. pilosa*.

Palabras clave
alelopatía • efecto estimulador • supresión de malezas

Introduction

The choice of cover plant is important for the success of crop rotation, with species used individually or in a consortium. The benefits provided by these plants include improvements in the physical properties of the soil, reduction of erosion, incorporation of nutrients, and suppression of weeds (12). However, plant coverage can also have an allelopathic effect that suppresses germination and inhibits the initial growth of plants of agricultural interest that are subsequently planted in the site (2, 6).

In southern Brazil, intercropping of cover crops such as grasses and legumes is a common and recommended practice. The consortium approach allows an intermediate carbon/nitrogen (C/N), which is not always possible with isolated species, and also results in a lower rate of decomposition than that of isolated legumes, thus providing longer soil coverage and synchronization between the supply and demand of nitrogen (N) for crops (25).

Among the cover crops used in summer, pearl millet (*Pennisetum glaucum* (L.) R.Br.) promotes strong nutrient accumulation and high phytomass production over a short period of time (19). *Crotalaria* (*Crotalaria spectabilis* Roth) is a legume recommended in combination with pearl millet that has a main advantage of reducing nematode populations (1). In the winter, black oats (*Avena strigosa* Schreb), a rustic plant with rapid growth, is the most cultivated in southern Brazil owing to its high dry matter yields and low N release speed from wastes. When this plant is applied in combination with leguminous or Brassica species, improved vegetation coverage in the soil and greater N fixation or recycling often occurs (7). The Brassicaceae forage radish (*Raphanus sativus* L.) has a high capacity for N extraction from deep soil layers, rapid initial development, and high dry matter yield (27), which makes it suitable for intercropping with black oats.

Crotalaria juncea and millet plants have been shown in the past decades to exert strong suppression of invasive plants while promoting high soil coverage and canopy light capture (8, 10, 16). In addition to the physical factors, chemical factors are also involved in the suppression of weeds by cover plants. For example, Hagemann *et al.* (2010) verified that the use of extracts from the aerial parts of white and black oats caused a reduction in the germination and root and hypocotyl growth of ryegrass and peanuts. Furthermore, studies on the use of allelochemical aqueous leaf extracts have been performed to mimic what occurs in nature (15, 22).

Considering the above, our aim was to investigate the potential allelopathic influence of intercropped or non-intercropped aqueous cover plant extracts on the physiological performance of soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) seeds, as well as their phytotoxicity to the hairy beggarticks (*Bidens pilosa* L.) weed.

Material and methods

The cover crops used were pearl millet [*Pennisetum glaucum* (L.) R. Br.], cultivar BRS 1501; *Crotalaria* (*Crotalaria spectabilis* Roth), black oats (*Avena strigosa* Schreb) cultivar EMBRAPA 29; and forage radish (*Raphanus sativus* L.) cultivar IPR 116. The seeds were purchased from a commercial supplier.

Plants were cultivated in a field in a rural area in the municipality of Terra Roxa, Parana State, Brazil (24°13'54.32" S, 53°58'46.90" W, altitude 303 m) to obtain the vegetal dry masses for aqueous extract preparation. The sowing period and quantity of seeds used followed the protocol described by Calegari (2016). Fertilization and weed, crop disease, and pest management had not yet been conducted.

The size of all production parcels was 10 m^2, and planting was performed by haul. The fall/winter cover plants, black oat and forage radish, were planted in combination and in isolation at the beginning of April 2018. When combined, 30 g (2068 seeds) of black oat + 5 g (455 seeds) of forage radish were used, while individually, 66 g (4550 seeds) of black oat and 16.2 g (1117 seeds) of radish were employed. The green mass was harvested 86 d after emergence, when both species were in the phenological stage of flowering.

Similarly, the spring/summer coverage crops *Crotalaria* and pearl millet were cultivated in consortium and individually at the beginning of October 2018. When in consortium, 10 g (570 seeds) of *Crotalaria* + 8 g (2000 seeds) of millet were used; when isolated, 20 g (5000 seeds) of millet and 15 g (857 seeds) of *Crotalaria* were utilized. During the phenological stage of flowering, 82 days after emergence, the green mass was harvested.

After biomass collection, the material was dehydrated in the shade with continuous passage of wind. The dry mass was then crushed in a blender and distilled water was added to obtain aqueous extracts at a concentration of 5%. Subsequently, the mixture was strained using a sieve.

To simulate the context of the first soybean crop and the second corn crop, aqueous extracts from the black oat and forage radish plants were used with the soybean seeds, and extracts from the *Crotalaria* and millet plants with the corn seeds.

The aqueous extract (5%) treatments for soybean seeds (Monsoy 6410) were as follows: black oat, forage radish, and their combination. Distilled water was used as the control. The test was conducted in accordance with the standards of the Rules for Seed Analysis (RSA) (17), consisting of eight replicates of 50 seeds. The evaluations were performed on the fifth and eighth days of the experiment to determine germination percentage and seedling length (cm).

Correspondingly, for corn (Pionner 3380), the aqueous extracts (5%) treatments were *Crotalaria*, pearl millet, and their combination. Distilled water was used as the control. The test was conducted in accordance with the standards of RSA (17), consisting of eight replicates of 50 seeds. On the fifth and eighth days of the experiment, germination percentage and seedling length (cm) were determined.

All aqueous extracts were assessed for germination of the hairy beggarticks (*Bidens pilosa* L.) weed. The tests were performed with five replicates of 50 seeds each. The seeds were placed on two Germitest papers in Gerbox type plastic boxes, which were stored in a BOD-type chamber at 25°C with a 14 h photoperiod. The Germitest papers were moistened with the extract in a proportion of 2.5 times the dry paper weight. Germination counts were performed simultaneously each day, with radicle emission by the seed as the determining factor. The germination percentage and germination speed index were calculated following the protocols of the Ministry of Agriculture, Livestock and Food Supply (MAPA), Brazil (2009) and Maguire (1962).

The results were analyzed using SISVAR software (9). Data were subjected to analysis of variance, and when significant differences were found, the Tukey test was used to compare the averages at 5%.

Results and discussion

The black oat and forage radish extracts cultivated both individually and in combination promoted gains in germination and length of soybean seedlings (table 1). This suggests that there was no undesirable chemical effect of straw (dry biomass) on the initial growth of the soybeans. This system is common in southern Brazil. Our results corroborate those of Krenchinski *et al.* (2018), who verified that the use of black oats, either isolated or intercropped with radish, had the potential to increase soybean yield.

Table 1. Seed germination percentage (%G) and seedling length (SL) of soybean in the presence forage aqueous extracts.

Tabla 1. Porcentaje de germinación de semillas (% G) y longitud de plántulas (SL) de soja en presencia de extractos acuosos de forraje.

Treatments	Variables	
	% G	SL (cm)
Control	93 b	14.68 c
Black oats extract	95 ab	17.51 b
Forage radish extract	97 a	17.88 b
Consortium extract	97 a	21.86 a
CV (%)	1.74	3.10

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

The effect of straw has also been reported on *Zea mays*, an important crop in Brazil, which is the second largest producer globally. Before harvest, a green manure system has also been proposed for some crops.

All cover plant extracts tested with corn promoted gains in seed germination; however, the extracts of isolated pearl millet and *Crotalaria* + millet combined reduced the length of the seedlings. Treatment with the isolated *Crotalaria* extract increased the growth of corn seedlings (table 2). According to Nunes *et al.* (2014), *Crotalaria* extract (*Crotalaria juncea*), compared to the other plants studied (*Brassica napus* L., *Crambe abyssinica* Hochst, *Linum usitatissimum* L., and *Raphanus sativus* L.), contributed the most to the increase in the germination percentage and growth of lettuce, cucumber, and soy seedlings. The positive effect of *Crotalaria* on the initial growth of corn indicates increased vigor of the plants. Carvalho *et al.* (2004) investigated *Crotalaria* grown in the spring and observed that this cover plant provided an 18.5% increase in corn productivity, compared to that of the fallow area. This increase in productivity may be correlated with a strong initial development of corn seedlings in the field.

Table 2. Seed germination percentage (% G) and seedling length (SL) of corn in the presence of forage aqueous extracts.

Tabla 2. Porcentaje de germinación de semillas (% G) y longitud de plántulas (SL) de maíz en presencia de extractos acuosos de forraje.

Treatments	Variables	
	% G	SL (cm)
Control	92 b	14.63 b
Pearl millet extract	95 a	12.93 c
Crotalaria extract	96 a	19.05 a
Consortium extract	97 a	13.69 c
CV (%)	1.57	4.23

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

All tested extracts suppressed germination and decreased the speed of germination index (SGI) of the hairy beggarticks when compared to the control (tables 3 and 4). Treatment with millet extract reduced the germination percentage by 47% and induced a considerable delay in germination. This extract also resulted in the shortest initial length of corn (table 2, page 73), which is likely related to the allelopathic effect of the cover plant, although *B. pilosa* was more sensitive to the presence of allelopathic compounds. The radish extract was the most phytotoxic for weed seeds, reducing germination by 89%.

Table 3. Percentage of germination (% G) and speed of germination index (SGI) of hairy beggarticks in the presence of cover plants aqueous extracts (pearl millet and crotalaria).

Tabla 3. Porcentaje de germinación (% G) y velocidad del índice de germinación (SGI) de la hierba *B. pilosa* en presencia de extractos acuosos de forraje (mijo perla y crotalaria).

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

Treatments	Variables	
	% G	SGI
Control	79 a	17.66 a
Pearl millet extract	42 c	6.87 d
Crotalaria extract	62 b	11.75 b
Consortium extract	49 c	8.66 c
CV (%)	4.25	5.60

Table 4. Percentage of germination (% G) and speed of germination index (SGI) of hairy beggarticks in the presence of cover plants aqueous extracts (black oats and forage radish).

Tabla 4. Porcentaje de germinación (% G) y velocidad del índice de germinación (SGI) de la hierba *B. pilosa* en presencia de extractos acuosos de forraje (Avena negra y rábano forrajero).

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

Treatments	Variables	
	% G	SGI
Control	78 a	17.39 a
Black oats extract	66 b	12.84 b
Forage radish extract	8 d	1.68 d
Consortium extract	48 c	8.63 c
CV (%)	8.13	8.59

Studies demonstrating the chemical and physical effects of cover crops of different species on invasive plants have been recurrent (5, 18, 21, 23, 26). Sturm *et al.* (2018) claimed that knowledge of the proportions of allelopathic and competitive effects could lead to the development of cover crop consortia with optimal weed suppression characteristics in the field.

Taken together, the results obtained herein demonstrate the benefit of using cover crops, a strategy that not only contributes to the integrated management of weeds, but also improves the production system by increasing the initial growth of soy and corn.

Conclusion

The aqueous leaf extracts of the two investigated plant consortia and the isolated cover plants favored the germination of the crops of interest, soy and corn, and were phytotoxic to noxious beggarticks. Forage radish extract most effectively reduced the germination of hairy beggartick seeds.

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Pre-germination treatments on *Ochetophila trinervis*, a native Andean tree with potential use for restoration

Germinación de semillas de *Ochetophila trinervis*, árbol nativo de los Andes Centrales con uso potencial de restauración

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Abstract

In a climate change scenario, global forest loss had a direct impact on the hydrological cycle, making the protection of soil and water resources a central issue. In the Central Andes of western Argentina, information on Chacay (*Ochetophila trinervis*) mountain forests is scarce. This tree thrives along river and stream banks, fixes atmospheric nitrogen, and grows in impoverished soils. The seeds of *O. trinervis* are characterized by physical or physiological dormancy, and germination requires technique application. The main goal was to evaluate the effect of mechanical and chemical scarification, cold stratification, and hot water immersion on the final germination percentage, germination speed index, and the mean germination time of *O. trinervis* seeds. Our results show that mechanical and chemical scarification are the treatments that best inhibit seed dormancy in this species. Mechanical scarification with sandpaper is the treatment that offers a balance between effective results and an easy-to-apply technique. Sulfuric acid (SA) treatment is also efficient in breaking dormancy, but we recommend applying it under extreme careful laboratory conditions.

Keywords
Rhamnaceae • watershed restoration • pre-germinative treatments • seed dormancy

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Resumen

La pérdida global de bosques en un escenario de cambio climático tiene un impacto directo en el ciclo hidrológico, por lo cual, la protección del suelo y los recursos hídricos adquieren una relevancia crucial. En los Andes Centrales del Oeste de Argentina, hay escasa información sobre los bosques de montaña, incluido el Chacay (*Ochetophila trinervis*), el cual se establece en bordes de ríos y arroyos, fija nitrógeno atmosférico y crece en suelos empobrecidos. Las semillas de *O. trinervis* presentan dormición física o fisiológica y la germinación es promovida mediante la aplicación de técnicas previas. El objetivo principal de nuestro estudio es evaluar el porcentaje final de germinación, el índice de velocidad de germinación y el tiempo medio de germinación en cuatro tratamientos (escarificación mecánica, escarificación química con ácido sulfúrico, estratificación fría, inmersión en agua caliente) y su control. Nuestros resultados muestran que la escarificación mecánica y química son los tratamientos que mejor inhiben la dormición de las semillas de esta especie. La escarificación mecánica con papel de lija es un tratamiento efectivo que ofrece un equilibrio entre los resultados obtenidos y una técnica sencilla de aplicación. Por otro lado, el uso de ácido sulfúrico presentó resultados óptimos de germinación, sin embargo, su aplicación debe realizarse en condiciones de laboratorio y con extremo cuidado.

Palabras clave

Rhamnaceae • restauración de cuencas • tratamientos pre-germinativos • dormición de semillas

Introduction

The global forest loss between 1990 and 2015 (3%) had a direct impact on the hydrological cycle, soil resources and provision of ecosystem services (6, 8). In the context of climate change and environmental hazards, the protection of soil and water resources becomes a central issue (13). In Argentina, land-use changes have caused annual deforestation rates of values between 200,000 ha (14) and 300,000 ha (6), which led to the sanction of the Ley de Bosques in 2007. As a result, it is necessary to develop guidelines for forest protection, focusing on distribution, conservation, and ecological restoration of the degraded areas (27).

In Mendoza province, Central-West Argentina, the information about the current distribution and conservation of mountain forests, which includes Maitén (*Maytenus boaria*), Luma (*Escallonia myrtoidea*), and Chacay (*Ochetophila trinervis*), is scarce (3). These woodlands play a critical role in river landscape conservation (15). Therefore, further studies are necessary to develop and implement restoration projects to reverse river use modifications and transformations related to village settlements.

Ochetophila trinervis (Gillies ex Hook. & Arn.) Poepp. ex Miers (Rhamnaceae) is a native South American tree of the Andes of Chile and western Argentina (22), found from 31° and 48° S, and between an altitude of 1,400 and 2,300 m (20). This species thrives along river and stream banks in the mountains (22), fixes atmospheric nitrogen and grows in impoverished soils (18). The study of the potential distribution of *O. trinervis* indicates that watercourses and related local conditions present optimal habitat suitability (24). However, there is evidence that these forests show geographical range retraction: historical sites such as the Chacay stream in the Uspallata valley exhibits signs of use and degradation (11).

Ecological features of *O. trinervis* highlighted this species as a suitable option for restoration projects due to its ability to stabilize watersheds and prevent flooding. Information on the environmental requirements for germination and on the eventual presence of dormancy in the seeds of focal species is a fundamental input for restoration tasks. This actinorhizal tree has dry fruits known as tricocos and small, hard seed shells (20, 22). Germination tests conducted in northern Patagonia reached 65% in previously stratified seeds (17).

Dormancy is a stage when a seed is unable to germinate under specific environmental conditions (2). This adaptive strategy allows germination to occur when suitable conditions are within the range of requirements for radicle emergence. Different species from arid environments present physical dormancy (5, 25, 26), including some species in the Rhamnaceae family (1), characterized by the presence of water-impermeable layers in the seeds (2). Dormancy interruption techniques attempt to simulate the ecological process that promotes germination, such as cold or winter conditions, mechanical abrasion from stream transport and acid softening in the digestive tract of animals during granivorous dispersal, among others. Physiological dormancy is regulated hormonally and requires cold stratification, whereas scarification is applied to break physical dormancy (1, 2).

Objective

Our objective was to evaluate the effect of mechanical and chemical scarification, cold stratification, and hot water immersion on the final germination percentage, germination speed index, and the mean germination time of *O. trinervis* seeds. We hypothesized that this species seeds present physiological or physical dormancy.

Materials and methods

Seed collection area

The fruit was collected in the early autumn of 2018 and 2019 on the eastern slope of Cordon del Plata, Central Andes of Mendoza, Argentina (32°59’45.12” S, 69° 15’58.85” W). In this area, the weather is semi-arid and local variability in the temperature and rainfall influences the distribution and abundance of vegetation (12), which adapts to climatic conditions of dryness and cold (4).

Experimental design

Fruit collection was carried out manually in five different forest patches located at least 250 m apart to ensure genetic diversity, including 30 individuals with healthy fruit in the surroundings of the Blanco River. Seeds were removed using a threshing device and stored at room temperature (18 ± 4°C) until evaluation. Broken and insect-damaged seeds were discarded by visual observation. Before each assay, the seeds were sterilized by immersion in commercial hypochlorite diluted at 10% for ten minutes and then washed three times in sterile water. Petri dishes of 9 cm diameter were used for the germination trials, with a cotton-wool layer over a filter paper disk. The experiment lasted 16 days, with four replicates of 25 seeds per treatment.

The following randomized treatments were defined: M= Mechanical scarification with sandpaper for 20 s and water immersion for 24 h; SA= Sulfuric acid scarification for 10 min; T5 °C= Cold stratification at 5°C for 15 days (16); T80 °C= 12 hours immersion with an initial temperature at 80°C; and Control (tC)=seeds without any treatment. The Petri dishes with the seeds were placed in a chamber (O.R.L. S.A Hornos Eléctricos) with temperature control at 25°C (±2°C) (16). Germination was defined and recorded as radical emergence to 1 mm. The sprouted seeds were counted daily for 16 days. Then, three parameters were estimated: final germination percentage, germination speed index, and mean germination time. Final germination percentage (%) was calculated as (n/N) × 100; where n is the number of germinated seeds, and N is the total number of seeds (6). Germination speed index (seeds day^{-1}) was estimated using the Maguire index (9) = $\Sigma_{(ni/ti)}$, and mean germination time (day^{-1}) was measured as = $\Sigma_{\,ni \times ti} / \Sigma ni$; where *ni* is the daily number of germinated seeds, and *ti* is the number of days spent on each count.

Data Analysis

Final germination percentage (%) was calculated as (n/N) × 100; where n is the number of germinated seeds, and N is the total number of seeds (7). Germination speed index (seeds day^{-1}) was estimated using the Maguire index (10) = $\Sigma_{(ni/ti)}$, and mean germination time (day^{-1}) was measured as = $\Sigma\, ni \times ti / \Sigma ni$; where *ni* is the daily number of germinated seeds, and *ti* is the number of days spent on each count.

Final germination percentage data were analyzed with generalized lineal models. Poisson distribution was used with log link function and the five levels treatment factor was considered as the fixed effect. Post hoc tests were performed using Tukey's HSD. All data were calculated with the GermCalc function from SeedCalc package (21) for R software (16).

Germination speed index and mean germination time data were normalized using a log10 transformation and analyzed with ANOVA, and LSD Fisher's test ($p < 0.05$) was used for means comparison. All figures present the original values of these three parameters.

Results

The applied treatments have significant effects on final germination percentages (LRT=292.58; Prob>Chi2 =2.2e-16), germination speed index (F value=24.74; p-value<0.0001), and mean germination time (F value=3.115; p-value=0.04). Final germination percentage values increased with M and SA treatments, exceeding the 50% germination value. These treatments presented the highest values of daily germination (figure 1a). The T80 °C treatment presented intermediate values of final germination percentage (22%±2.6). The T5 °C treatment reached a low germination percentage (13%±3), the values achieved were similar to the control treatment (8%±2.8) (figure 1b).

Each letter indicates significant differences ($p < 0.05$, Tukey test).

Cada letra indica diferencias significativas ($p < 0,05$, prueba de Tukey).

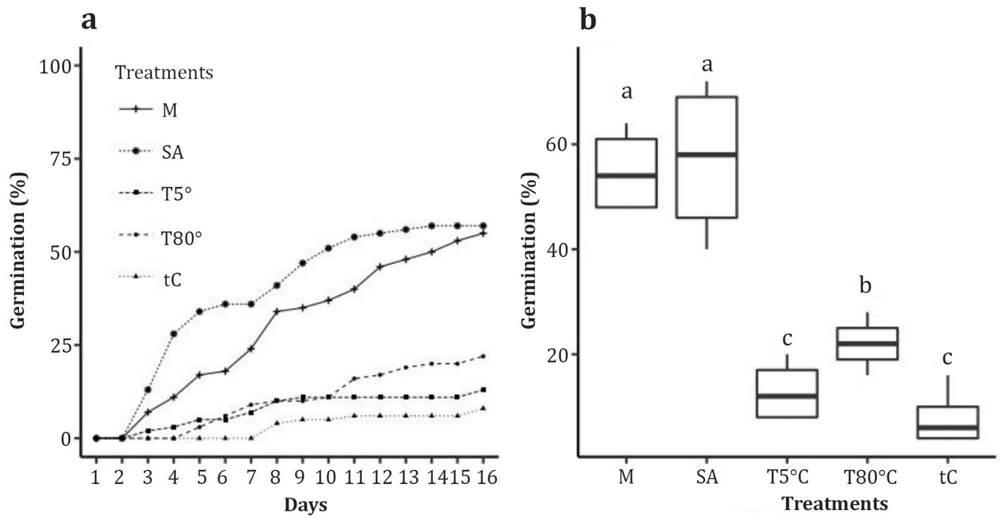

Figure 1 (a). Cumulative germination percentage and **(b)** final germination percentage (FGP) of *Ochetophila trinervis* seeds under different treatments (mechanical (M), sulfuric acid (SA), temperature at 5°C and 80°C (T5°C and T80°C, respectively) and control (tC).

Figura 1 (a). Porcentaje de germinación acumulada y **(b)** porcentaje final de germinación (PFG) de semillas de *Ochetophila trinervis* bajo diferentes tratamientos (mecánico (M), ácido sulfúrico (SA), temperatura a 5°C y 80°C (T5°C y T80°C, respectivamente) y control (tC).

M and SA treatments showed the highest values for the germination speed index, with a rate between 2.1 and 3 seeds per day (figure 2a, page 81). The temperature treatments (T5°C and T80°C) presented intermediate values for the germination speed index, with 0.6 and 0.7 germinated seeds per day, respectively. The SA treatment was the one that reached the shortest mean germination time (6 days ±0.2). Although these results are different from those obtained in the temperature treatments (T5°C and T80°C) and control (tC), they do not differ significantly from the values obtained in the M treatment (figure 2b, page 81).

Each letter indicates significant differences ($p < 0.05$, Fisher-LSD).

Cada letra indica diferencias significativas ($p < 0,05$, Fisher-LSD).

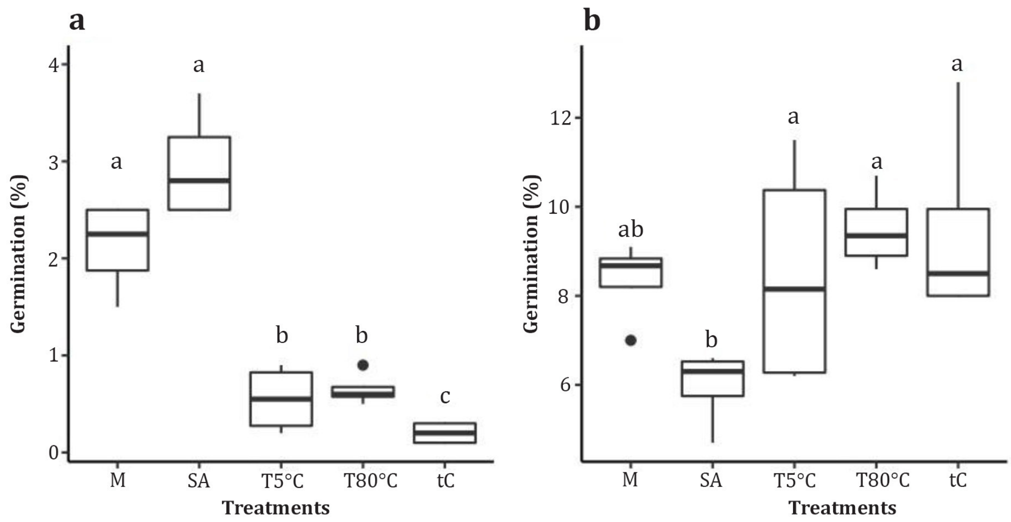

Figure 2(a). Germination speed index (GSI) and **(b)** mean germination time (MGT) of *Ochetophila trinervis* seeds under different pre-germination treatments (mechanical (M), sulfuric acid (SA), temperature at 5°C and 80°C (T5 °C and T80 °C, respectively) and control (tC).

Figura 2 (a). Índice de velocidad de germinación (IVG) y **(b)** tiempo medio de germinación (TMG) de las semillas de *Ochetophila trinervis* bajo diferentes tratamientos pre-germinativos (mecánico (M), ácido sulfúrico (SA), temperatura a 5°C y 80°C (T5 °C y T80 °C, respectivamente) y control (tC).

Discussion and conclusions

Our findings support the hypothesis that the seeds of *O. trinervis* show physical dormancy because either mechanical (M) or chemical scarification (SA) techniques achieved the highest germination values. The hot water immersion treatment (T80°C) performs poorly, similar to trials on other Rhamnaceae species from Australia (23). Regarding the stratified procedure (T5°C), our results show the lowest germination values, similar to the control treatment (tC). According to this outcome, seed dispersal is likely to occur through transport by streams or by ingestion by wildlife.

The cold stratification treatment (T5°C) seems viable for northwest Patagonia (17), where lower temperatures than in our study area can induce a winter pause and *O. trinervis* seeds do not need scarification to achieve a high germination rate. This observation differs from our results and the procedures applied to *Discaria tomatou*, a Rhamnaceae species from New Zealand, where scarification techniques also promote higher germination rates (9). The differences between each experiment could be explained by climate contrast between the study sites, the seed dispersal mechanisms and would imply a high interspecific variability.

In this study, we seek to adjust a simple and effective technique for the germination of *O. trinervis*, a tree with forestation potential and watershed restoration capacity. Our results indicate that mechanical scarification (M) might be a practical option for seedling germination in the Central Andes of Western Argentina. Sulfuric acid (SA) treatment is also efficient in breaking dormancy, but we recommend applying it under extreme careful laboratory conditions. Further study should focus on seed viability and other environmental factors that potentially regulate the germination process, such as temperature, humidity and light exposure.

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Resilience and performance of wine cooperatives in Castilla La-Mancha (Spain) during a period of financial crisis

Resiliencia y desempeño de las Bodegas Cooperativas de Castilla La Mancha (España) en periodo de crisis

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Abstract

The economic crisis has had an asymmetric effect on Spanish and regional/local economies. This study aims to analyze the strategies developed by cooperative wineries in Castilla-La Mancha (CLM) and their impact on performance measurements. The paper opted for an exploratory study based on a compilation of financial statements consisting of the traditional economic-financial profitability ratios (ROA, ROI, ROS) plus a specific analysis, Return of Owner Cooperative (ROC). We have also used two financial measurements: Liquidity and Leverage. Trade dynamism is the hallmark of wine cooperatives in CLM in terms of strategic action in the face of a crisis. Their resilience is patent in the conquest of foreign markets via low unit costs, which have been transferred to sales prices. The paper has implications for the understanding of the resilience of the agricultural cooperatives during the crisis period in question. The consistency of the results provides a context to promote the cooperative model as an essential factor in the social economy. This paper fulfils an identified need to show the cooperative model as a resilient one in the agricultural field and in the context of rural development.

Keywords

cooperatives • wine • resilience • financial crisis • Spain

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Resumen

La crisis económica ha tenido un efecto asimétrico en las economías españolas a nivel nacional y regional/local. Este estudio analiza las estrategias desarrolladas por las bodegas cooperativas de Castilla-La Mancha (CLM) y su impacto en las medidas de rendimiento. Se ha optado por un estudio exploratorio basado en una compilación de estados financieros, se ha definido como medidas de rendimiento los tradicionales ratios de rentabilidad económico-financiera (ROA, ROI, ROS) y adicionalmente un análisis específico: Rendimiento para el socio cooperativista (ROC). También se han incluido dos ratios financieros: Liquidez y Apalancamiento. El dinamismo comercial es el sello distintivo de las cooperativas vitivinícolas de CLM como acción estratégica ante la crisis, y su resiliencia es patente en la conquista de mercados exteriores a través de bajos costes unitarios, que se han trasladado a los precios de venta. En este trabajo se incluyen las implicaciones para la comprensión de la resiliencia de las bodegas cooperativas durante el período de crisis. La coherencia de los resultados proporciona un contexto para promover el modelo cooperativo, como factor esencial de la economía social. Este documento responde a una necesidad identificada de mostrar el modelo cooperativo como el modelo más resistente en el ámbito agrícola y como dinamizador en el desarrollo rural.

Palabras clave

cooperativas • vino • resiliencia • crisis económica • España

Introduction

The Spanish wine sector is immersed in a constant process of change because of increased competition in an increasingly global and international market. Castilla La Mancha (Spain) is the largest wine-growing region in terms of surface area in the European Union, so knowledge of the competitiveness of its wineries is a significant factor. This region has a unique feature compared to other wine-producing regions, which is the cooperative sector's enormous weight. This balance between private wineries and cooperatives justifies the importance of this study.

In the period analyzed (2002-2011), two milestones have had a substantial though unequal impact on the wineries. Firstly, the industry has undergone a significant restructuring because of a legal transformation in the Common Market Organization (CMO) and secondly, the financial crisis after 2007. The wine cooperatives have made substantial efforts in terms of strategies. Cooperative integration and the food chain law (Law 12/2013, August 2) and the creation of the *Interprofesional del vino* [Spanish inter-professional wine organization, 2014)], have also entailed regulatory changes and incentives for the modernization of business processes and inter-sector relations, and the relationship between value chains structures.

To cope with the post-2007 economic crisis, the cooperatives opted for financial caution, cost reduction based on lower settlements to partners (self-financing) and above all, for the establishment of second-degree cooperatives. Baco is a second-degree cooperative consisting of eight first-degree wine coops and had a turnover volume of 40 million euros in 2011. It merged with DCOOP (olive oil, Andalusia) in a cooperative integration process under *Ley 13/2013 de Fomento de la Integración Asociativa* [Association Promotion Law]). This involved large bulk volumes and the need to export at much more competitive price levels compared to other world production areas. Nonetheless, it is necessary to highlight the small size of the CLM cooperatives even after the merger compared to their French and Italian counterparts.

The purpose of this paper is to study whether there exist differences in the wine cooperatives' performance as a result of the significant sector transformation. The reaction of wine cooperatives and the strategies they developed were substantially different from those adopted by private wineries. They former used distinctive characteristics of the cooperative movement, which allowed them to survive, adapt and find solutions during changing situations in the context of an economic crisis (from 2007) or the modification of the CMO (13).

The organization of this paper is as follows. First, we present the wine sector in Castilla-La-Mancha, the most significant region in production terms, and its context. Second, we describe the competitiveness of the wineries and their efforts to capture a competitive advantage and survive in a period of financial crisis. Third, the database and the methodology used and fourth, the results. Finally, we draw some conclusions and recommendations.

The wine sector in Castilla-La-Mancha (Spain)

Castilla-La-Mancha - CLM is the most significant wine region in Spain and the E.U. It sells the largest volume of wine and must. Table 1 shows that CLM represents 9.7% of Spanish wineries and sold 56% of the nation's wine volume in 2014-2019.

CLM hosts 218 wine cooperatives out of a total number of 425 in Spain. Some of these wine cooperatives are larger than private wineries (IOF) in terms of average firm size. In 2013, only 27 out of the 580 wineries produced more than 50,000 hl, and almost 70% (19) of these 27 wineries were cooperative associations (16).

Wine cooperatives in CLM base their competitive position on winegrowers carrying out intensive farming, which provides a much higher average yield than the national mean. It is also essential to remember that wine cooperatives represent 67.5% of the vineyard area at the regional level (32).

Table 1. Importance of the wine sector in Castilla-La-Mancha (Spain).

Tabla 1. Relevancia del sector vitivinícola en Castilla-La-Mancha (España).

Economic information	Spain	CLM		
		Total	IOF	Coop
Wineries (nº) 2018	4,373	425	207	218
Volume of wine (miles hl) (Average 2014-2019)	43,390	24,400	9,500	14,890
Turnover (2017) Mill. €	6,958	1,878	1088	790
Average yield hl/ha (Average 2009-2014)	42.55	49.51	n.a.	51.07
Export 2018				
mill hl	25.4	13,3		
mill €	3,288	890,4		
Prices (€/litre)	1.29	0.67		

Source: (23, 27, 32, 33) and authors/ Fuente: (23, 27, 32, 33) y elaboración propia.

In terms of volume of exports, CLM represents 52.4 % of total Spanish exports. However, it only reaches 27.1% of the Spanish total in terms of value. This situation shows a specialization in large bulk volumes, in which cooperatives are the main actors (37). The export volume from CLM set a record with 15.1 million Hl commercialized in 2017 in international markets; only 25% of the wine produced remained in the Spanish domestic market in the form of distillations (byproducts and potable alcohol) and must. The main destinations for CLM's wine are EU countries -and due to the border effect- France, Portugal, Italy, and Germany. CLM wine's low average export price is significant: 0.67€/l in CLM *vs.* a Spanish median of 1.29€/l (32).

Airén is the main grape variety in CLM. It is considered a prime example of a bulk winemaking grape, and it is closely associated with regional cooperative production. It fell in hectares cultivated from 338,000 (2000-2007 average) to 215,000 in 2013 while unit yields increased exponentially to produce record harvests in 2013. The fact that wine-growing holdings have had access to significant budget aid to start vineyard reconversion and restructuring irrigation and trellis systems has also been a determining factor. Regarding the CMO Restructuring and Conversion of Vineyards, CLM invested 842 million euros during the 2001-2012 period (more than 50% of the funding allocated to Spain for this end), and no fewer than 130,000 hectares were converted out of a total of 465,000 throughout the region (38). Wine cooperatives represent approximately 50% of AOC area wine sales (8.5 million hl). However, cooperatives have a high level of efficiency in comparison with non-cooperative wineries (13).

Competitiveness in the wine sector: measurement indexes and hypotheses

The contribution of this paper is to identify different profiles of wine cooperatives and show their performance in order to understand their financial strengths. When performance is analyzed, we consider companies' operating and financial results measured based on accounting variables.

Arguments for and against using different strategies to compete in the market are related to typologies of cooperatives. Based on previous papers (10, 11, 19, 21, 35, 44), we identified different strategies used by cooperatives to be resilient to changes arising from the financial crisis and changes in legal regulations.

Social economy companies withstood the crisis better due to their objective function (maximizing cooperative owners' income) and thanks to their flexibility in terms of grape payment policies (1, 24, 41).

The importance of economic performance as a result indicator is unquestionable, and it is in this light that we analyze company survival and solvency (17, 25). Traditional profitability ratios such as ROA or ROI do not reflect cooperatives' objectives. We propose another ratio, Return of Cooperative Member (ROC), comparing these measures to assess performance (12, 43).

Performance aside, the economic crisis has also substantially changed companies' financial situation. Therefore, adopting resilience strategies has a direct effect on liquidity and debt indicators (3). Namiki, 2013 analyzes liquidity and leverage. In terms of leverage, Amendola *et al.* 2012 (correlated the debt ratio of Italian companies with lower indebtedness. Other studies (19) compared cooperatives and private businesses in Italy. They conclude that cooperatives based their emergence out of the crisis on higher levels of solvency and efficiency, as opposed to private companies, which mainly used their availability of liquidity as a shock strategy through the economic depression stage. The case of Spain highlights the export outlet as a factor for a notable increase in cooperative sales despite the barriers to cooperative internationalization (22).

Therefore, and based on the previous variables, we intend to test the hypotheses below, designed to control for variations in performance.

Environmental variables

Environmental and context changes have modified the competition rules in the sector. Studies related to wineries' economic-financial situation focus their attention on the strategies developed in the countries of research in "Old Europe." In Italy, wine cooperatives' need to adapt is associated with globalization (8). Portuguese wine cooperatives are facing difficulties in operating in a more competitive global market. They need to change their policy of maximum return to partners as this severely reduces their capacity to improve their debt structure and finance long-term investment (39). Studies on the Languedoc-Roussillon region in France point out the need to reconcile the maximization of grape payment to partners typical of the cooperative model with the need to generate reserves for long-term investment (40). Studies comparing Austria, Germany, and Italy (2) or France and Italy (15) distinguish cooperatives focusing on bulk sales and those focusing on the sale of bottled wine and conclude that diversification and segmentation are the strategies with the highest positive effects in terms of performance.

Economic development allows for studying large wine producers in developing countries. A differential factor was analyzed in Argentina, namely, whether cooperative owners obtain better prices for their raw material from non-associated producers (31). The case of Chile highlights the importance of cooperatives, even though three large companies dominate the Chilean market (29). Nguyen *et al.* (2013) analyzed growth and crisis processes in Australia, as did Cordery and Sinclair (2013) in New Zealand.

A common thread across these studies claims that wine cooperatives have systematically and dynamically adapted to changing world situations and to the regulatory and structural changes required by different scenarios to gain a competitive position and sustainability. Above all, it shows that the evolution of the wine sector in “Old Europe” countries cannot be understood without considering the cooperative sector’s fundamental role.

The primary purpose of this research is to analyze the resilience of cooperatives in the largest wine-producing region in a context of economic crisis and significant changes in public regulation, and the implications for their performance measures.

H1: There are no differences in Performance and Financial Situation due to environmental variables

Variables such as firm size and age modify ways of competing. From a theoretical point of view, large companies adapt better to environmental changes, so size has a positive effect on performance and financial situation measurements (42).

H1-1: There are no differences in Performance and Financial Situation due to size

Second, we formulated a hypothesis indicating that age affects performance and financial situation measurements. The effects of age on these measurements are contradictory. Age has a negative correlation with profitability in Gardebroek *et al.* (2010). By contrast, recent studies reveal a positive correlation derived from movements on the average costs curve (30), although previous studies showed results in the opposite direction (18). The debate about age leads to the following hypothesis:

H1-2: There are no differences in Performance and Financial Situation due to age

Commercial strategies

Commercial strategies modify ways of competing. Environmental changes and increased competition due to changes in the CMO and the production from emerging markets have forced cooperatives to alter their marketing strategies (6, 15, 27).

H2: There are no differences in Performance and Financial Situation due to commercial strategies

One of the variables to consider is sales development. Increased competition levels and the fall in per capita consumption create marketing problems in wine trading. Added to this, an increase in average yield per hectare generates even more difficulty. In this context of competition, a fall in consumption and an economic crisis, sustaining a positive trend in sales is vital. Some studies correlate sales increase with ROA (9). This relation would entail that apart from quantity, average sales price also allows the recovery of costs and produce a profit margin.

H2-1: There are no differences in Performance and Financial Situation due to Growth of Sales

A further commercial strategy adopted by wineries is exporting. Companies positioning themselves abroad has led to the sale of significant volumes in new foreign markets. One of the reasons for the previous lack of presence on international markets is bulk commercialization instead of selling bottled wine. New investments in bottling, new packaging options (Bag in Box), and a competitive price policy has allowed for positioning and sales in non-traditional markets. Exports are related to the positive impact on the development of wine companies (5, 33).

H2-2: There are no differences in Performance and Financial Situation due to Export activity

Modernization through investment has enabled cooperatives to make improvements in terms of production and commercialization. This investment, in some cases intensive in nature, was financed by EAFRD (European Agricultural Fund for Rural Development) Grants. Some studies (7) record the level of EAFRD aid to wine cooperatives in terms of support to quality promotion investment.

H3: There are no differences in Performance and Financial Situation due to ERDF Grants

The rejection of the proposed hypotheses would allow us to state that the adoption of different strategies affects cooperatives' economic performance and financial situation.

Materials and methods

We will test these hypotheses using a sample made up of financial statements of wine cooperatives (CNAE code 1102: winemaking) in CLM (Spain) recorded in the *Registro de Cooperativas* (Public Registrar's Office). We created a longitudinal dataset by building a sector sample covering the 2002 -2011 period.

The original database included 45 cooperatives active in 2001; hence five coops were eliminated. The final database (40 cooperatives) is an unbalanced panel representing 18.3% of the wine cooperatives in CLM. However, if we consider the sales volume for 2011, our sample represents 23% of the cooperative sector and 18% of wine production in CLM.

This sample does not present problems in terms of the significance of the results. The sample error represents 6% at a 95% confidence level according to the estimation of error for finite populations and taking the assigned turnover for 2011 as the reference variable.

The bulk of the cooperatives in the sample are small (micro and small following E.U. criteria (20). The definition of variables was carried out based on the hypotheses formulated in the previous section, and they are either continuous or discrete (dummy) in nature (table 2).

Table 2. Variables.

Tabla 2. Variables.

Variables	Typology	Description
Performance measures		
ROA	Continuous	Return on Assets = (Profits before interest and taxes)/Total Assets
ROI	Continuous	Return on investment = (Profits before interest expenses and taxes plus interest income)/Total Equity
ROS	Continuous	Return of Sales = (Profits before interest and taxes)/Total Sales
ROC	Continuous	Return of Cooperative-Owner = (Purchases / Sales)
Environmental		
SIZE (A)[(a)]	Dummy	1 if the firm micro or small firm; 0 otherwise
AGE	Dummy	Year of creation the cooperative, before the 70s 1; 0 otherwise
Commercial		
SALESGR	Continuous	Growth of Sales: (Sales $_{it}$ - Sales $_{it-1}$) / Sales $_{i,t-1}$
EXP	Dummy	1, if the firm exports; 0, otherwise
Financial		
EAFRD Grants	Dummy	1, if the firm receives EAFRD Funds; 0, otherwise
LIQ	Continuous	Liquidity = (Current Assets/ Current Liabilities)
LEV	Continuous	Leverage (Total Assets - Own resources) / Own resources

Source: Authors.

Fuente: Elaboración propia.

Table 3 shows the descriptive statistics of the variables analyzed. This information shows that the 2007 crisis resulted in negative values for all performance measurements. The ROC variable, for instance, has a meagre minimum value, which indicates that the grape price paid to partners is also low. Therefore, the income received by cooperative members transfers the problem from the organization to winegrowers.

Table 3. Descriptive analysis.

Tabla 3. Análisis descriptivos.

	Descriptive	N	Min	Max	Mean	Standard Deviation
Continuous variables	ROA	324	-1.99	10.2	0.14	0.64
	ROI	324	-16.46	47.13	0.56	3.29
	ROS	324	-1.74	0.92	0.11	0.34
	ROC	324	0.01	2.62	0.72	0.35
	LIQ	324	-1.01	28.47	1.37	1.92
	LEV	324	-84.07	1.176.43	5.95	66.83
	SALESGR	284	-1	266.07	1.344	13.17
	Frequencies	N	0	1		
Dummy variables	Size	324	50	274		
	Age	324	77	247		
	EXP	324	131	193		
	ERDF Funds	324	287	37		

Source: Authors.

Fuente: Elaboración propia.

The indicators corresponding to the financial situation (Liquidity and Leverage) highlight the impact of the crisis, showing both a lack of short-term liquidity and an increase in indebtedness in this period.

The bulk of the cooperatives in the sample consists of micro and small cooperatives (85%). This percentage is similar to that of the Spanish industrial world, in which smaller companies are predominant.

The policies carried out in the 1960s-1970s, and the transition to democracy favored the creation of cooperatives, reaching 76% of cooperatives in this period. Between 2008 and 2015, the number of companies in the beverage manufacturing sector fell 10% in Spain, whereas in CLM, it fell only 3%. The cooperative sector has grown both in presence and activity level in this sector (28).

Cooperatives increased sales despite the crisis in the period studied. This fact highlights their resilience during crisis periods and the efforts made in terms of market orientation through increases in sales.

Export orientation (EXP) reveals the importance of the efforts made by cooperatives to expand into foreign markets. The lifting of distillation measures entailed a massive transformation of wineries as they needed to sell wine for distillation. 60% of the wineries in our sample sell part of their products in foreign markets. Finally, only 12% of cooperatives received EAFRD grants. This aid supported investment in the period analyzed.

The methodology used to test H1-H3 reflects the static aspects of cooperatives. It requires year on year comparison of the means and variances of each of the different variables across environment, commercial and financial situations using a standard t-test of the difference of two means and an F-test of equality of two variances between any two populations. Note that the term "no differences" in H1-H3 has two meanings, and the interpretations of the results presented in the next section will reflect that. The first meaning refers to its standard explanation, namely that the variable in question does or does not have the same value across the criteria considered. Hence, the test is one of equality *versus* non-equality of means. The second measures the alternative hypothesis and deals with the particular degree of profitability for the variable tested below that of their counterparts due to the specific nature of strategies. We test both cases by comparing the p-value of each test to a Type I error, α, of 0.1, and the following decision rule.

Decision rule

If (p-value)>α, conclude that there are no differences in the characteristic in question as a result of differences in variable considered.

If p-value<α/2, conclude that there are differences in the characteristic in question because the value of the said index is lower than for the counterpart.

If α/2≤p-value<α, conclude that there are differences in the characteristic in question, but not necessarily because the value of the said index is lower than that for the counterpart.

Results and discussion

Competitiveness and strategies developed by wineries

Table 4 shows the evolution of performance measurements over time. Based on this information, it is safe to state that despite the crisis, wine cooperatives have maintained positive mean values in performance measurements, which supports cooperatives' resilience within the different stages of the economic cycle.

Table 4. Evolution of performance measurements mean values.

Tabla 4. Evolución de las medidas de desempeño y valores medios.

Mean	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Δ	2002-2006 (%)	2007-2011 (%)
ROA	0.48	0.03	0.06	0.08	0.15	0.17	0.16	0.11	0.14	0.06	-0.87	-0.69	-0.63
ROI	1.02	0.01	0.22	0.26	0.5	0.61	0.99	0.27	1.56	-0.11	-1.11	-0.51	-1.18
ROS	0.05	0	0.09	0.1	0.16	0.21	0.15	0.14	0.11	0.04	-0.3	2	-0.82
ROC	0.86	0.88	0.79	0.74	0.68	0.63	0.69	0.63	0.67	0.78	-0.1	-0.21	0.24
LIQ	1.28	1.25	1.64	1.43	1.13	1.31	1.21	1.38	1.23	1.8	0.4	-0.12	0.37
LEV	1.73	1.93	2.19	2.28	3.82	2.9	-0.72	1.38	6.72	33.3	18.2	1.2	10.5

Source: Authors. Fuente: Elaboración propia.

However, if we analyze the global impact, all performance variables fell in the period studied. Let us consider the economic cycle in stages. We see that during the financial boom period, the performance indicators reflected negative values if we consider the traditional measures. It is worth noting that the ROS does not follow this pattern in this period, given that between 2002 and 2006, growth is positive (2.00) and that it became negative during the crisis (2007-2011). These results show that traditional performance measurements do not reflect the actual profitability level of cooperatives. There is, therefore, an accounting measurement problem in that it does not show the main objective of a cooperative measured as the transfer of income to cooperative owners.

Cooperative owner income fell in the overall period. However, if we observe its evolution by sub-periods, the situation is the opposite of that expected; the growth of the ROC is negative in the economic boom period, whereas it shows positive values during the crisis.

This result deserves special attention and will be discussed in subsequent sections. The financial variables reflect a variation in the liquidity ratio. During the period 2002-2006, this ratio was negative, probably related to the increase of the investment ratios of the wineries and, for the period 2007-2012 the liquidity increased 0.37% due to the austerity measures developed by the coops. There was also an increase in the debt ratio in the period 2007-2001, where the ratio growth was 10.5%.

Table 5 (page 92-93) shows this analysis results. The results include the median values of the significant variables based on the preceding approach represented as mean values for the grouping variable.

Table 5. p-values of means and means of the significant index.
Tabla 5. Valores p de medias de los índices analizados.

			Mean of the index																			
			2002	2003		2004		2005		2006		2007		2008		2009		2010		2011	
Environment	Size	ROA	0.54	0.03		0.05		0.04		0.11		0.13		0.09	*	0.09		0.12		0.07	
			-0.05	0.02		0.09		0.29		0.30		0.34		0.44		0.23		0.23		0.01	
		ROI	1.14	0.04		0.19		0.20		0.36		0.42		0.83		0.17		1.65		-0.13	
			-0.14	-0.18		0.36		0.62		1.14		1.32		1.61		0.92		0.94		-0.02	
		ROS	0.07	0.02		0.07		0.06		0.11		0.17		0.09	*	0.11		0.09		0.05	
			-0.06	-0.12		0.19		0.38		0.38		0.35		0.41		0.35		0.26		-0.03	
		ROC	0.85	0.85		0.80		0.77		0.72		0.65		0.75		0.66		0.69		0.77	
			1.00	1.07		0.75		0.56		0.51		0.54		0.45		0.44		0.56		0.87	
		LIQ	1.31	1.28		1.73		1.49		1.22	*	1.44		1.31		1.42		1.26		1.93	
			1.02	1.05		1.10		1.08		0.75		0.85		0.84		1.08		1.03		0.96	
		LEV	1.73	1.71		2.02		2.16		2.70		2.45	*	-1.84		1.03		7.18		38.05	
			1.72	3.46		3.20		3.04		8.86		4.52		3.61		3.78		3.63		3.82	
	Age	ROA	2.57	0.01		0.03		-0.01		0.04		0.11		0.13		-0.02	**	-0.03	**	-0.07	
			0.02	0.03		0.07		0.10		0.18		0.19		0.17		0.16		0.19		0.11	
		ROI	5.54	0.00		0.05		-0.02	*	-0.03	*	0.26		0.85		-0.54		0.20		0.07	
			0.02	0.01		0.27		0.34		0.64		0.72		1.04		0.55		2.03		-0.17	
		ROS	0.24	0.02		0.04		-0.01	*	-0.06	*	0.13		0.12		-0.09		-0.04		-0.16	**
			0.01	-0.01		0.10		0.14		0.22		0.23		0.16		0.21		0.17		0.10	
		ROC	0.70	0.77		0.77		0.75		0.86		0.66		0.69		0.81	*	0.79		0.93	*
			0.90	0.91		0.80		0.74		0.63		0.62		0.69		0.57		0.63		0.73	
		LIQ	1.38	1.96		1.94		1.53		1.47		2.10		1.41		1.71		1.46		4.21	
			1.26	1.05		1.55		1.40		1.04		1.05		1.14		1.26		1.16		0.99	
		LEV	1.09	0.97		0.81	**	1.42	*	4.39		2.95		-7.21		-1.94		-2.69		-0.47	
			1.88	2.20		2.59		2.55		3.67		2.88		1.52		2.53		9.97		44.54	
Commercial strategies	Sales Growth	ROA		-0.06		0.07		0.10		0.14		0.18		0.17		0.12		0.06		-0.09	
				0.05		0.06		0.06		0.15		0.17		0.14		0.08		0.21		0.12	
		ROI		-0.28	**	0.27		0.23		0.53		0.65		0.29		0.37	**	0.19		0.14	
				0.07		0.24		0.32		0.32		0.63		1.12		-1.42		2.86		-0.21	
		ROS		-0.15		0.11		0.14		0.18		0.24		0.20		0.15		0.07		-0.18	
				0.03		0.10		0.08		0.15		0.18		0.13		0.03		0.15		0.12	
		ROC		1.04		0.78		0.72		0.68		0.51		0.61		0.61		0.66		0.98	
				0.88		0.78		0.77		0.68		0.71		0.72		0.82		0.68		0.70	
		LIQ		1.11		1.25		1.21		1.14		1.84		1.25		1.45		1.03		1.17	
				1.12		1.42		1.65		1.24		1.10		1.15		0.68		1.43		2.04	
		LEV		2.19		2.28		2.18		2.48		2.05		3.15		2.29		1.60		-2.27	
				1.97		2.35		2.25		3.25		2.46		-1.57		-15		11.6		46.97	
	Export	ROA	0.00	0.01		0.00	*	-0.05	**	0.05		0.12		0.23		0.08		0.0	**	0.17	
			0.82	0.05		0.11		0.17		0.21		0.21		0.11		0.13		0.2		0.00	
		ROI	-0.02	-0.01		-0.04	*	-0.10	**	0.23		0.50		1.95	*	0.23		0.0		0.47	
			1.75	0.02		0.42		0.51		0.68		0.68		0.32		0.30		2.5		-0.48	
		ROS	0.00	0.01		0.00	*	-0.05	**	-0.02	**	0.16		0.20		0.08		0.0	**	0.12	
			0.09	0.00		0.16		0.21		0.28		0.24		0.12		0.18		0.2		-0.01	
		ROC	0.92	0.84		0.85		0.85		0.85	**	0.66		0.65		0.70		0.8	**	0.73	
			0.83	0.91		0.75		0.66		0.57		0.61		0.72		0.59		0.6		0.81	
		LIQ	1.55	1.51		2.26		1.85		1.22		1.70		1.40		2.01		1.8	*	3.10	
			1.10	1.05		1.14		1.14		1.07		1.07		1.08		0.93		0.9		0.97	
		LEV	1.67	1.62		1.79		1.91		2.36		3.17		-5.38		-1.07	*	0.7		1.00	
			1.78	2.18		2.51		2.54		4.76		2.73		2.52		3.09		10.5		53.84	

Investment	ERDF Grants	ROA												0.26		0.11		0.13		0.10	
														0.02		0.10		0.15		-0.02	
		ROI												1.02		0.26		1.79		0.46	
														0.95		0.34		0.50		-1.42	
		ROS												0.25		0.13		0.10		0.07	
														0.02		0.22		0.17		-0.04	
		ROC												0.58	*	0.64		0.69		0.74	
														0.85		0.60		0.58		0.86	
		LIQ												1.31		1.41		1.33		2.12	
														1.06		0.97		0.80		1.07	
		LEV												-1.54		1.31		8.70		0.17	
														0.46		2.27		-2.33		10.57	

** Significance 5% (p-value). * Significance 10% (p-value).

Source: Authors. Fuente: Elaboración propia.

Environmental variables

The weight of the size and age variables in accounting for performance measurements is uneven and concentrated in time. Thus, if we consider cooperatives' average size, we see that it is not significant in the period studied. Even though they can go in both directions, the differences are concentrated in the 2008 financial year. 2008 is the year of the CMO (Common Market Organization) wine reform and the onset of the economic crisis. In this case, small wine cooperatives obtained worse results in terms of performance than medium-sized and large ones.

In terms of age, the bulk of cooperatives were established in the 1970s which provides them with expertise and stability. Older cooperatives should show better performance. However, we were not able to verify this conclusion given that as from 2005 results tend to be contradictory.

Commercial strategies

Changes in wine cooperatives' structure and sales strategies have resulted in their transformation. These changes have in turn resulted from the new regulation of CMO and the creation of a new AOC in the region with a differentiated commercial strategy.

The first variable analyzed is sales growth. Our underlying assumption would be that wine cooperatives with a positive evolution of sales should show better performance indicators. These differences in favor of cooperatives with bigger sales volumes were concentrated between 2009 and 2011 when cooperatives with positive sales had better performance.

The second strategy adopted by wine cooperatives was foreign market expansion. The Spanish market shows falling rates of wine consumption, so cooperatives have had to opt for exporting to sell their wine. The underlying assumption was that there are no differences in performance measurements due to export orientation. The data in the corresponding table shows that the export strategy improved performance measurements in the period studied, particularly in the 2004-2010 period.

From a financial point of view, our results reveal mean differences, although the sign of the mean values is not conclusive given that liquidity falls and leverage increases. Foreign market sales have reduced wine cooperatives' liquidity and increased debt to finance these liquidity problems.

Investment and CAP Policy

Here we will consider the variable corresponding to the impact of ERDF Grants on performance measurements.

In the E.U. framework, co-funding investment strategies carried out by companies in preferential areas are the target of financial support. Access to these grants materialized from 2008 when cooperatives with this source of funding obtained better performance indicators. However, these grants did not substantially modify performance measurements after 2008, probably due to the economic crisis.

Conclusions and further research

The importance of wine cooperatives in Castilla-La Mancha (Spain) is unquestionable. It is the region with the largest cultivated area and is one of the world's top five wine-producing areas. However, its financial results do not match this leading position, neither measured in liters nor in terms of the wineries' performance. It is thus of particular importance to analyze this phenomenon.

Wine cooperatives have in the last few years transformed themselves in terms of strategies and market orientation, and this has been as the result of two factors: first, the lifting of distillation measures has forced them towards market orientation to facilitate wine commercialization, and second, the economic crisis significantly affected this sector. Both wine consumption and price per litre fell.

Considering the results of this study, the cooperatives of Castilla-La Mancha have shown themselves to be resilient actors in the wake of the economic crisis, globalization, and changes in European public regulation. They have opened new export markets to counteract the decline of domestic consumption and the disappearance of the market grants for distillation. They have consolidated performance rates despite the widespread crisis scenario in the European economies. These results further show the causes of this behaviour, which coincide with earlier results obtained in European countries: France, Italy, Portugal, as well as in other producing countries: Argentina or Chile.

This analysis reveals those environmental variables unevenly affected performance measurements: Size does not appear relevant in this sector framework, whereas age allows access to better competitive positions based on cooperatives' expertise.

From a commercial point of view, sales strategies have the most positive impact or have at least not been too detrimental due to the economic crisis. Cooperatives have survived in the market in times of crisis. Many of them show a positive trend in sales growth that has allowed them to differentiate themselves from the rest of the companies in terms of performance measurements, especially since 2009. Export orientation has also improved performance. This is the strategy with the highest impact over the whole period of study, and it is safe to state that non-exporting companies show, in many cases, negative values in performance measurements.

Finally, it is worth noting that the economic crisis has prevented the expected takeoff in terms of demand for EAFRD funds. Differences were observed only in the first year; they have not been sustained over time. The volume invested by cooperatives has slowed down because of the economic crisis.

In this context, wine cooperatives have had to reinvent themselves. They have emerged as agents resilient to the effects of the crisis; against all odds, they have maintained and even improved their performance indicators. This work has provided food for thought in understanding the strategies and adaptive capacity developed by cooperatives facing the financial crisis and the CMO Law reform. As for future lines of research, it may be possible to assess the study of these strategies and resilience to non-financial crises such as Covid-19.

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Multicriteria decision analysis for fruits and vegetables routes based on the food miles concept

Análisis de decisiones multicriterio para rutas de frutas y verduras basado en el concepto de millas de alimentos

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Abstract

The aim of this research is to propose a performance evaluation system to rank the efficiency of fruit and vegetables (FV) distribution routes based on selected criteria related to logistic, distribution practices and physical losses that are part of the attributes associated with the food miles concept. To achieve this end, the multicriteria Preference Ranking Method for Enrichment Evaluation (PROMETHEE) was adopted. The distance that a food item travels from the field to the table is an important logistical indicator and sooner it arrives in the hands of the consumer, the greater the degree of preservation of quality, *i.e.*, the integrity of the item's sensory and organoleptic characteristics. An analysis of the logistics involved should take into consideration issues such as distance, transportation time, pollutant emissions, conditioning, and all the other attributes pertinent to the food miles concept. Short routes were the most efficient, however the perishability of the product was not the main factor, this is because aspects related to a good ability to offer products, transport them safely and assertiveness in predicting demand contributed more to the high efficiency rates, thereby, it is the short routes that service the predilections of consumers who value locally produced food.

Keywords

logistical performance • food miles • PROMETHEE • wholesaling • food distribution • transport • MCDA • food supply chain

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Resumen

El objetivo de esta investigación es proponer un sistema de evaluación del desempeño para clasificar la eficiencia de las rutas de distribución de frutas y hortalizas (FV) con base en criterios seleccionados relacionados con la logística, las prácticas de distribución y las pérdidas físicas que forman parte de los atributos asociados al concepto de millas de alimentos. Para lograr este fin, se adoptó el Método de Clasificación de Preferencias multicriterio para la Evaluación del Enriquecimiento (PROMETHEE). La distancia que recorre un alimento desde el campo hasta la mesa es un indicador logístico importante y cuanto antes llegue a manos del consumidor, mayor es el grado de conservación de la calidad, es decir, la integridad de las características sensoriales y organolépticas del producto. Un análisis de la logística involucrada debe tomar en consideración aspectos como la distancia, el tiempo de transporte, las emisiones contaminantes, el acondicionamiento y todos los demás atributos pertinentes al concepto de millas de alimentos. Las rutas cortas fueron las más eficientes, sin embargo la perecibilidad del producto no fue el factor principal, esto se debe a que aspectos relacionados con una buena capacidad para ofrecer productos, transportarlos con seguridad y asertividad en la predicción de la demanda contribuyeron más a las altas tasas de eficiencia, por lo que, son las rutas cortas las que sirven a las preferencias de los consumidores que valoran los alimentos producidos localmente.

Palabras clave

rendimiento logístico • millas de alimentos • PROMETHEE • venta al por mayor • distribucion de comida • transporte • MCDA • cadena de suministro de alimentos

Introduction

Although substantial there is much research available addressing growth and the importance of local production and consumption to for the development of sustainable agriculture is now available, this research contributes in the analysis of the wholesalinge sector of fruits and vegetables (FV) role in this innovative new vision of production and consumption. Cities have come to rely heavily on large scale food sourcing (7), giving rise to new logistical issues, since the globalizing of food production systems requires long haul travel for its transportation (42).

In the USA, for example, the average distance traveled by fresh produce from the farm to the consumer market has increased in recent decades, currently ranging between 1,500 and 2,500 miles (22). In contrast, in the European Union, products travel an average of 865.5 miles (32).

In Brazil, the fruit and vegetable logistical supply chain (FV) makes little use of best distribution and supply practices. Among other factors, long distance transportation hampered by an inefficient logistics system with prolonged journey times, trucks with no refrigeration, and ineffective packaging, all contribute to the deterioration of product quality, loss and waste (45).

The hypothesis of this study is that food consumption based on economic logic is insufficient to promote the efficiency of agrifood chains, as the mitigation of losses is also emerging and the promotion of sustainable logistic systems.

The aim of this research is to propose a performance evaluation system to rank the efficiency of fruit and vegetables (FV) distribution routes based on selected criteria related to logistic, distribution practices and physical losses that are part of the attributes associated with the food miles concept. To achieve this end, the routes of FV marketed in a wholesale food market were evaluated using a multicriteria method the Preference Ranking Method for Enrichment Evaluation (PROMETHEE).

The application of decision-making methods to complex problems characterized by multiple criteriais on the increase (17). Several Multi-Criteria Decision Analysis (MCDA) methods can now be found in the literature, for example: Analytic Hierarchy Process (AHP), Data Envelopment Analysis (DEA), Elimination and Choice Translating Reality (ELECTRE), Multi-attribute Utility Theory (MAUT), Preference Ranking Method for Enrichment Evaluation (PROMETHEE), Technique for the Order of Prioritisation by Similarity to Ideal Solution (TOPSIS), among many others (55).

MCDA methods can be classified into three categories according to their preference modeling principles: 1- synthesis of single criterion approach, 2- interactive judgment approach and 3- outranking approach (35). In the case of outranking methods, there is a construction of an overclassification/supremacy relationship, which in-corporates the preferences established by the decision maker (28, 57). It was within this infrastructure of outranking methods that PROMETHEE was developed (28).

The application of PROMETHEE and hybrid methods that include you, were used to assess the performance of supply chains, in particular regarding the strategies for locating distribution centers by (2, 24, 27, 47, 51). The multicriteria method has also been used to identification of strengths and weaknesses of attributes related to food security (46), selection of green suppliers in the field of the food supply chain (21) and organizational performance management applied to fruit exporters (48).

The food miles concept fits this perspective as it addresses the importance of production close to the place of consumption, the concept has ramifications that go far beyond local food in its consideration of environmental, economic and social sustainability issues. The importance of food miles is such that they can be considered an indicator of sustainable development (40). Research discussing the importance of the food miles concept is now extensive in the literature (37, 42).

Food distribution connects consumption to production in different ways (20). This is especially so in the fresh produce wholesaling sector (6). In Brazil, two companies, CEASA Campinas and CEAGESP, are considered references in the Wholesale Food Market, in addition to being important players in the supply of several Brazilian municipalities. CEASA Campinas sells robust volumes of horticultural products despite being located in the same state as the largest centralized supplier in the country (54).

To this end, the fruit and vegetable routes marketed by the wholesaling center, CEASA Campinas, were evaluated using PROMETHEE. The FVs were chosen accordingly from the most commercialized lines in terms of volume and/or number of financial transactions.

Materials and methods

PROMETHEE is one of the most recent MCDA methods developed by Brans in 1982 and in the years following several modules were created and applied to the most diverse of areas (4, 11, 25, 28, 31, 37, 57). The method is based on the comparison of pairs of alternatives ac-cording to each criterion, and in relation to the weightings. The result is a ranking of the options from the best to the worst, *i.e.*, based on the construction of an overclassification relationship (10, 57). The main difference that separates PROMETHEE from other methods is that it considers the internal relationships of each item in the assessment during the decision-making process (1, 10, 37) and advantage stems from its mathematical properties and easy interaction with the user (1, 35).

The steps necessary to apply the method are. First, define a decision matrix containing a set of alternatives and criteria: The Multicriteria problems solved with PROMETHEE consist of a finite set A, of n alternatives where f1 to fk are k criteria. The fj (a) function is the evaluation of the alternative "a" in the fj criterion (9). The data is expressed in a preference matrix format. Each row corresponds to an alternative and each column to a criterion. Whenever not declared, it is assumed that all criteria fell into the earned value category (21, 37). Set preferences for each of the criteria: First, the decision maker must assign weightings "w" to each criterion, according to their relative importance. The higher the value of the weighting, the greater the importance of the criterion (4, 29).

$$\sum_{j=i}^{k} wj = 1$$

Next, the decision maker can decide whether the weighting will be maximized or minimized so as to achieve optimization (10, 11). When a criterion is maximized, this function gives preference to "a" over "b" for deviations observed between criteria evaluations (10).

Finally, the decision maker selects the preference function that is to be associated with each criterion. Rj (a, b) represents the function of the difference between the evaluations of alternative "a" compared to alternative "b" in each criterion in a degree ranging between 0 and 1. The dj (a, b) refers to the assessments of "a" and "b" in each criterion (1).

$$Rj\ (a,b) = Fj\ [dj\ (a,b)]$$

The selection method is enriched by six possible forms of preference function: 1- usual criterion (no parameters are defined); 2- U-shape (parameter q is defined); 3- V-shaped criterion (parameter p is defined); 4- level criterion (parameters q and p are defined); 5- V shape with in-difference criterion (q, e, p parameters are defined) and 6- Gaussian criterion (standard deviation must be fixed) (37). Once the preferences have been set (weightings, maximizing/minimizing and the preference function), the next step in PROMETHEE modeling is to compare each action against each other. This is done by calculating a multicriteria preference index (9).

$$\{\pi(a,b) = \sum_{j=1}^{k} \quad Qj(a,b)wj\ \pi(b,a) = \sum_{j=1}^{k} \quad Qj(a,b)wj$$

The degree of exceeding π (a, b) expresses to what degree "a" is preferable to "b" over all criteria; and, π (b, a) as "b" is preferable to "a". Both π (a, b) and π (b, a) are normally positive values. The following properties are valid:

$$\{\pi(a,a) = \ 0, 0 \leq \pi(a,b) \leq 1, 0 \leq \pi(b,a) \leq 1, 0 \leq \pi(a,b) + \ \pi(b,a) \leq 1$$

π $(a, b) \approx 0$ indicates that a is WEAKLY preferable to b

π $(a, b) \approx 1$ indicates that a is STRONGLY preferable to b

Calculate the important flows: Each alternative competes with (n-1) and other alternatives. The balance of these competitions can be expressed through two flows, positive and negative. It should be emphasized that the greater the net flow, the better the performance of the alternative.

The net preference flow is the balance between positive and negative preference flows:

Positive flow (Phi +) ($ø^{+}$) of overshoot: $ø^{+}(a) = \frac{1}{n-1}\sum_{x\in A} \quad \pi(a,x)$

Negative flow (Phi-) (ø-) of overshoot: $ø^{-}(a) = \frac{1}{n-1}\sum_{x\in A} \quad \pi(x,a)$

From the information on net flows (Phi-) (ø-), the rankings of the alternatives are obtained in decreasing order of their respective flows.

The alternatives are first compared in pairs with each criterion according to the preferences of the decision maker, resulting in local scores which are then added to the global scores through the application of the PROMETHEE I and PROMETHEE II modules (31). The basic principle of PROMETHEE II (full ranking) is based on the net preference flow (Phi), which can be interpreted as a score attributed to the alternative according to its performance. It includes preferences and indifference: <P, I> (9). An alternative "a" can outperform an alternative "b" if the net flow of "a" is greater than the net flow of "b". The alternative "a" may be in-different to "b" if its net flows are the same. Thus, the ranking of alternatives is based on the decreasing order of their respective net flows (9). The GAIA module is an extension of the PROMETHEE results through a visual procedure, which helps in the interpretation of the results (10).

Description of the data

Five FVs were selected, which are among the most commercialized in terms of volume and/or number of financial transactions, sources of regular supply throughout the year in their respective categories and different perishables such as lettuce (*Lactuca sativa* L.) (leaf, flower and stem), tomato (*Lycopersicon esculentum* Mill.) (fruit), potato (*Solanum tuberosum* L.) (root, bulb, tuber and rhizome), orange (*Citrus sinensis* L. Osbeck) (fruit) and papaya (*Carica papaya* L.) (fruit) (14). Whatsmore, those produce is among those most commonly consumed by the Brazilian population, according to the latest Household Budget Survey - POF 2017-2018. In the group of fruits, orange ranks 3rd, followed by papaya in 4th, in the group of vegetables, tomatoes lead the ranking in 1st, potatoes in 2nd and lettuce in 8th (12).

The data were collected from the Brazilian Horticulture Market Modernization Program (Prohort) of CONAB (2019) and considered the following: road transport routes (from the city of production to CEASA Campinas), in addition to their respective volumes (in kg) and average annual value. Distances (in km) and time (in minutes) were also calculated. Field research was carried out in which wholesale traders were interviewed via a questionnaire. This questionnaire was applied on the spot between October 2019 and January 2020.

This methodological approach is known as rapid appraisal and has been applied (5, 18) and, with a focus on strategies for supply chain management, Alidrisi (2021) and Sukati *et al.* (2012). This method uses data from secondary sources together with interviews based on semi-structured questionnaires, in which detailed data and/or information are needed for understanding the dynamics of the sector evaluated. The development of the questionnaire was adapted from the work of Gustavsson *et al.* (2013) and Mendonça Lima; Ramos de Oliveira (2021) and reflects the logistical practices already established for each product category with a view to monitoring a sampling so as to ensure the representation would be appropriate (33). The number of wholesalers that sell exclusively lettuce, potatoes, oranges, papaya and tomatoes totals 81 wholesalers. CEASA Campinas wholesalers agreed to participate in the survey (87.6% response rate).

CEASA Campinas is an important Wholesale Food Market of fruits and vegetables and other fresh products for the state of São Paulo, being responsible for supplying more than 500 municipalities. There are more than 580 wholesalers, distributed in about 940 stores (15). The study limit is the Wholesale Food Market de Campinas and was chosen because of: (i) easy access and collection of merchant information; (ii) in the 2017-2019 triennium, 1.8 million tons of food were sold, of these, 560 thousand tons of fruits and vegetables, ranking as the eighth largest wholesaler in the country (16).

Selection of routes by distance range

The distances for all the routes analyzed were surveyed. Next, the routes were grouped into three distance ranges for each selected FV, classified as short, medium or long (table 1). For this division, the data was separated into a cluster using the SPSS software program (19).

Table 1. Categories of routes.

Tabla 1. Categorías de rutas.

	Lettuce	Potato	Orange	Papaya	Tomato
Categories of routes	**Distances (km)**				
Short	< 80	<350	< 210	< 610	< 400
Medium	80 - 200	350 - 1,200	210 - 1,100	610 - 1,800	400 - 1,550
Long	> 200	> 1,200	> 1,100	> 1,800	> 1,550

Criteria selected for evaluation

The criteria were established based on research that addressed the concept of food miles (44, 53). The concept also contributed to establishing the criteria, and identifying road transport practices, obtained from an interview with CEASA Campinas' FV marketing managers, as well as certain players in the wholesaling sector and transportation operators (15). In addition, the criteria were defined by their association with the quality of the product, or rather, attributes that should be configured to ensure the integrity of the food (maintenance of the physical and nutritional quality of food). Altogether, 12 criteria were defined as follows: 1- Distance, 2- Time, 3- Volume, 4- CO_2, 5- Price, 6- Losses, 7- Truck, 8- Packaging, 9- Own transport, 10- Traceability, 11- Change in packaging and 12- Handling. The calculation used for each criterion is described in supplementary material.

Weightings assigned to the criteria

The weighting assigned to each criterion was determined by a questionnaire applied to specialists in the sector (decision makers), who had to rank them in order of importance taking into account their perceptions of the food miles concept. Each specialist evaluated the criteria individually, and assigned percentages (again individually) whereby the sum of all of them totalled 100%. Immediately after, the arithmetic mean of the weighting of each criterion was calculated, as well as work developed (1). The definition of weightings by consultation with a specialist is a very common practice (1, 21). The usual criterion preference function was selected as being the most appropriate approach to the problem.

Results and discussion

The importance of measuring the logistical performance of distribution channels of different lengths (km) based on criteria supported by the concept of food miles and in the main logistical practices of distribution of fruits and vegetables, contribute to the definition of strategies to mitigate food losses and the promotion of more sustainable supply chains. This is one of the main contributions of this research, which also points out the vulnerable points of each distribution channel and proposes measures to be implemented to overcome these obstacles.

It can be seen that the short lettuce route alternative scored as being the most efficient within the established parameters (table 2). This result corroborates the tendency of consumers to value locally produced food, as has been shown firstly, by the work of Grebitus *et al.* (2013) in which the products analyzed with the shortest distance covered had the highest preference score with consumers and secondly, by the work carried out by Pektaş *et al.* (2017) who showed the extent to which degree of loyalty of people rose when valuing organizations that sell locally produced food. The alternative long papaya route, characterized by long journey and travel time, was the most efficient in the lower stratum compared to the long tomato, long potato and long orange routes, which covered much shorter distances. This is due to the fact that long papaya is transported in refrigerated trucks, as shown by Liu *et al.* (2020) where temperature can be a more effective factor in the preservation of fresh fruit and vegetables. What stands out is the significant difference between the short lettuce and medium lettuce scores, which produced a result of 57.47.

Table 2. Ranking of FV routes.

Tabla 2. Clasificación de rutas FV.

Routes	Score	Phi	Phi+	Phi-
LettuceS	100.0	0.5592	0.5902	0.0310
OrangeS	55.2	0.3226	0.4716	0.1490
PotatoS	46.3	0.2416	0.4445	0.2029
LettuceA	42.5	0.2014	0.4395	0.2381
PapayaS	41.6	0.1904	0.4157	0.2252
TomatoS	35.6	0.1152	0.4137	0.2986
OrangeA	30.7	0.0411	0.3502	0.3090
LettuceL	26.0	- 0.0419	0.2033	0.2452
PotatoA	22.4	-0.1165	0.2654	0.3819
PapayaA	21.2	-0.1440	0.2443	0.3883
PapayaL	19.6	-0.1808	0.2326	0.4135
TomatoL	18.6	-0.2075	0.1205	0.3280
PotatoL	15.5	-0.2933	0.0776	0.3709
TomatoA	13.9	-0.3400	0.1862	0.5262
OrangeL	13.7	-0.3475	0.0505	0.3980

The criteria of volume, packaging and traceability were the criteria that most penalized the alternative route. It is also important to note the influence of the criteria on each alternative (figure 1).

Phi+ means how much one alternative is dominating the others and Phi- means how much one alternative is dominating the others. The difference between these flows generates the Phi that shows the ordering of the alternatives, from the largest net flow to the smallest.

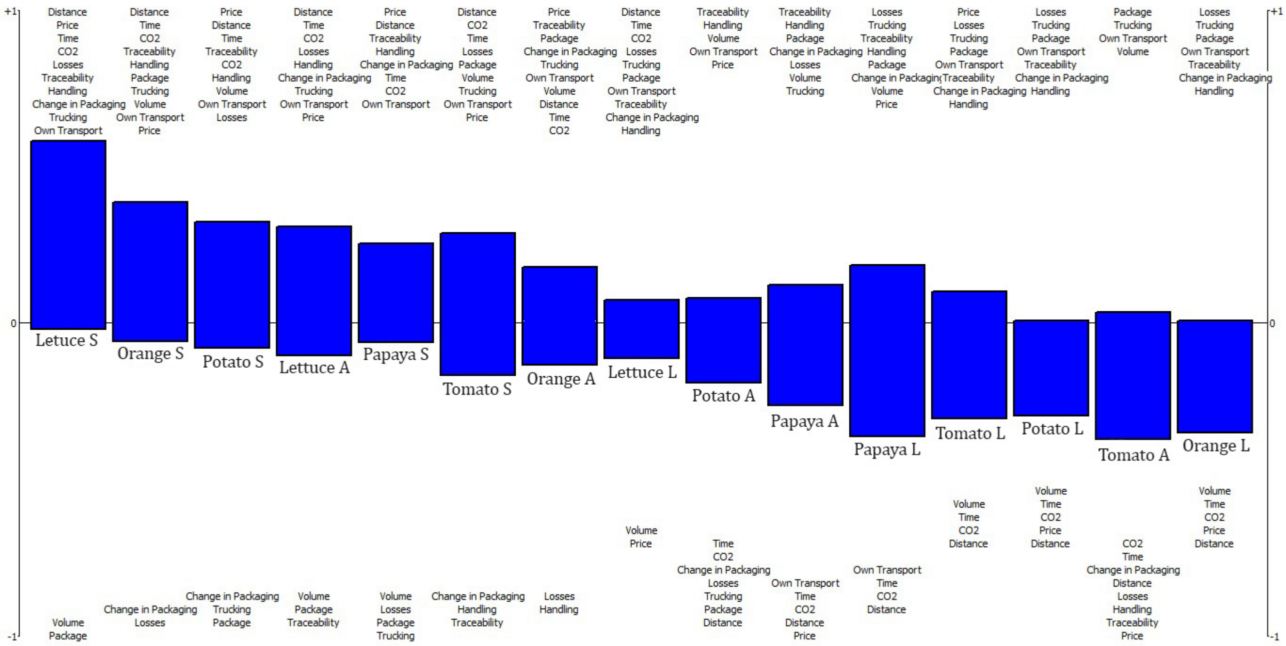

Figure 1. Participation of the criteria in the FV routes.

Figura 1. Participación de los criterios en las rutas FV.

The short lettuce route, which was the most efficient, presented the shortest distance from the field to the wholesaling center. However, the criteria of volume and type of packaging used showed negative results in the analysis (figure 1). Although the short lettuce route was the most efficient among the other routes, it did not meet 100% efficiency for all the criteria. This shows that even though it was the best route, it is still possible to find vulnerable points where improvements could be implemented.

Lettuce covers an average radius of 168 km as it is a very perishable food production that takes place closer to the wholesale center. Transport is more frequent and volumes are smaller, making the offer more assertive to meet demand, avoiding losses. On the other hand, papaya travels on average a radius of 1,517 km added with negative performance operational criteria contributed to greater losses. This result is proven by the work of Mendonça Lima and Ramos de Oliveira (2021) who disclosed that practices such as transshipment operation, transport of an open truck (often covered with canvas) and the use of "K" type packaging (wood) were associated with higher levels of residues for papaya.

In Slovenia, a survey of tomatoes showed that regional production around 250 km could reduce the ecological footprint left by transportation by up to 83.33%, compared to the 1,500 km transcontinental transportation journey (49). In Canada, a study analyzed the distances that imported foods travel and CO_2 emissions in 2006 showed that 30% of agricultural commodities consumed in the country were imported and that food miles exceeded 61 billion tons of km generating approximately 3.3 million tons of CO_2. Fruit and vegetables were, in fact, the foods responsible for emitting the most CO_2 (25).

In the case of tomato routes, the average route proved to be less efficient compared to the long route. The criteria of changes in packaging, losses, handling, traceability and price penalized the performance of the alternative medium route compared to the long route (figure 1, page 103). While short routes are more efficient, principally as a function of the distance involved, there are weak aspects to be overcome by better planning such as the type of packaging used and handling in relation to changes in packaging.

Weightings have a significant influence on performance analysis and the multicriteria model was associated with this problematic model, in the same way as the work carried out by Wu *et al.* (2020) in which the application of PROMETHEE proved it presented the best method for comparing alternatives.

The influence of the criteria, especially qualitative ones, on the analysis is significant. Research involving qualitative issues almost always raises doubts as to what constitutes best decision-making, and the use of multicriteria analysis in these cases is an efficient tool (21).

Overall analysis (figure 2) is based on the Phi flow (PROMETHEE II), which facilitates an ordering of the alternatives. When applying the module, an overall assessment is presented by overrun, whereby the short lettuce route is highlighted, followed by the second best alternative, which is the short orange route. The upper half of the scale (in green) corresponds to Phi + scores and the lower half (in red) to Phi- scores.

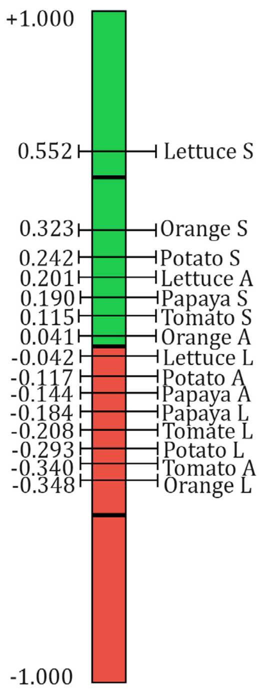

Figure 2. Overall ranking.

Figura 2. Clasificación general.

Once the product is harvested, it is no longer possible to improve its quality just be preserve, the use of refrigerated transportation is an important attribute because under conditions of monitored temperatures it is possible to travel a greater distance without materially penalizing product quality. However, from the perspective of sustainability, is not always the best, since ultimately, kilometers driven translate into greater emission of pollutants and higher economic costs. The use of more appropriate packaging for each type of FV and less handling should contribute to improvements in this configuration. The packaging's beneficial effects in maintaining produce quality during transportation and retail marketing have been demonstrated for many types of FV, such as mangoes, strawberries and kale (13). Edible coatings and waxes, based on proteins, lipids, polysaccharides and their composites, are also protecting and preventing food losses and waste (52). Cardboard packaging, for example, is sustainable, and post-first time use is then recycled. Furthermore, the fact that this packaging model supports lower volumes reduces the need to switch to lesser options, as with potatoes that arrive in 50 kg bags and in some cases when unloading, are transferred to 25 kg bags, or in the case of tomatoes, which are in plastic boxes and sometimes transferred to plastic bags. According to Müller and Schmid (2019), depending on the packaging, biological, chemical or physical processes can occur, leading to deterioration in the product.

The use of the best logistical models can be found on both long and short routes. Thus, distance and time become paramount when choosing the best routes. This contributes to meeting the expectations of new consumption trends whereby sustainability is prioritized.

In Brazil, logistics for FV products is still under development, since many of the operations are in deficit. As can be seen in the results of this research, certain medium and long routes may be more efficient as appropriate means of transport, packaging and refrigeration are deployed.

Relocating producing regions close to large consumer centers is not simple. This is because in Brazil the northeast region has proved to be quite suitable for the production of various FV, due to good climate and soil conditions. In addition, the northeast region is closer to the EUA and Europe, which contributes to shorter distances for export. The improvement of some practices can bring substantial gains to the sector before heavy investments in infrastructure.

A number of local marketing practices connecting producers and consumers have been adopted worldwide which, increasingly, are appearing in the Brazilian scenario; for example, the Community Supported Agriculture (CSA), known as the CSA (37). Although this model has proven to be quite well-structured and developed, there are others in the making gaining adherents. Such is the case for urban agriculture, where a community comes together to produce food in idle urban spaces, such as squares, backyards, balconies, vacant lots, public or private green areas, building slabs and shopping malls (38).

Although CEASAs has been gradually losing market share to large retail chains, the system remains crucial to the Brazilian model. However, the growth of populations looking for locally produced food calls for modernization of this system to meet the demands of the new consumer market. Considering the continental dimensions of Brazil, wholesaling centers should continue to maintain the interface between producers and consumers, since there are regions with potential for greater production of certain products the results can contribute to direct public policies on the promotion of sustainable foods and loss and waste reduction.

Porat *et al.* (2018) argue that measures, such as the public awareness campaigns and information platforms to publish and advertise adequate professionally recommended storage instructions are important policies soft. But it is also necessary that governments and municipalities can adopt more rigorous approaches in terms of public policy regulating the type and conditions of transport, more adequate storage and ways to avoid losses and waste, failure to comply with these measures can result in fines and fees. Such propositions have already been suggested in the studies by Bloom (2010) and Priefer *et al.* (2016), as pointed out Porat *et al.* (2018).

Conclusion

Short routes were the most efficient, however the perishability of the product was not the main factor this is because aspects related to a good ability to offer products, transport them safely and assertiveness in predicting demand contributed more to the high efficiency rates, thereby, it is the short routes that service the predilections of consumers who value locally produced food.

The food miles concept, as a starting point for evaluating routes, showed that the distance attribute is important, however, it is not the only one for defining the most efficient route, since the evaluation of the performance of the routes is multidimensional, considering operational aspects and also sales management, such as environmental impact, packaging, prices, distances and losses.

It is noteworthy that the adoption of short routes is not always the best alternative, as there are scenarios in which the reallocation of agricultural production would not be possible, due to land and climate. However, the adoption of good logistical practices associated with the concept is a positive tool for better performance on long routes.

One of the advances of this research was to show the contribution of the food miles concept to propose a performance evaluation system to classify the efficiency of fruit and vegetable distribution routes based on logistical criteria that go beyond distance.

This work may serve as a resource for future studies which seek points of improvement for less efficient routes. Once the attributes that need improvement are met, it is expected that the result will be greater preservation of sensory and organoleptic quality and less losses and waste, thus improving the supply of FV.

Supplementary material

https://drive.google.com/file/d/1wql_1-ECKoJXkrxO6_pjwlTzGxzCbjVP/view?usp=sharing

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Is *Taeniothrips inconsequens* (Thysanoptera: Thripidae) a pest of stone and pip fruit trees in Argentina?

¿Es *Taeniothrips inconsequens* (Thysanoptera: Thripidae) una plaga de frutales de carozo y de pepita en la Argentina?

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Abstract

The presence of the "pear thrips" *Taeniothrips inconsequens* has been cited in Argentina in 1921 by Teresa Joan. This has affected exports of fruit tree propagation materials. However currently there is a concern about that citation because it was probably an incorrect identification of the thrips species. The objective of this work was to confirm the presence of *Taeniothrips inconsequens* in fruit orchards in Argentina. Fruit orchards were sampled in the main producing areas of Argentina. A total of 10,696 specimens from 393 samples were examined and no *T. inconsequens* were found. The citation of this species for Argentina could be the result of misidentifications. Ninety percent of the collected specimens corresponded to four species of thrips: 37% *Frankliniella australis*, 29% *Thrips tabaci*, 14% *Frankliniella occidentalis* and 10% *Frankliniella gemina*. Of the remaining 10%, 2% were larvae and 8% corresponded to the species *Aneristothrips rostratus*, *Frankliniella frumenti*, *Frankliniella schultzei*, *Frankliniella inesae*, *Frankliniella juancarlosi*, *Frankliniella* spp, *Leptothrips mali*, *Aeolothrips fasciatipennis*, *Arorathrips texanus*, *Tenothrips frici*, *Haplothrips* spp, *Haplothrips fiebrigi*, *Haplothrips trellesi*, *Thrips australis*, *Karnyothrips* spp., and *Caliothrips phaseoli*.

Keywords
pear thrips • stone fruits • pome fruits • detection • pest

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Resumen

La presencia del "trips del peral" *Taeniothrips inconsequens* ha sido mencionada en el año 1921 por Teresa Joan. Esto afectó la exportación de material de propagación. Sin embargo, existen dudas sobre si la identificación de la especie fue correctamente realizada. El objetivo de este trabajo fue verificar la presencia de *Taeniothrips inconsequens* en Argentina y para esto se muestrearon distintos montes frutales en las principales zonas productoras. Se examinaron un total de 10696 ejemplares desde 393 muestras, y no se encontró *T. inconsequens*. La cita de esta especie para Argentina podría ser el resultado de identificaciones erróneas. El 90% de los especímenes recolectados correspondió a cuatro especies de trips: 37% *Frankliniella australis*, 29% *Thrips tabaci*, 14% *Frankliniella occidentalis* y 10% *Frankliniella gemina*. Del 10% restante, 2% fueron larvas y el 8% correspondió a las especies *Aneristothrips rostratus*, *Frankliniella frumenti*, *Frankliniella schultzei*, *Frankliniella inesae*, *Frankliniella juancarlosi*, *Frankliniella* spp, *Leptothrips mali Aeolothrips fasciatipennis*, *Arorathrips texanus*, *Tenothrips frici*, *Haplothrips* spp, *Haplothrips fiebrigi*, *Haplothrips trellesi*, *Thrips australis*, *Karnyothrips* spp. y *Caliothrips phaseoli*.

Palabras clave
trips del peral • frutales de carozo • frutales de pepita • detección • plaga

Introduction

Thrips are small, highly thigmotactic insects. Females of the suborder Terebrantia introduce their eggs in petioles, stems, leaves, and fruits, and therefore rapid visual detection is extremely difficult (11, 13). The "pear thrips" *Taeniothrips inconsequens* (Uzel) economically affects exports of stone fruit propagation materials. Then, certain requirements for the phytosanitary certification of shipments of apricot propagation material from Argentina to other countries such as Peru were required (18).

The pear thrips has a wide range of host plants, stone and pip fruit trees, as well as ornamental and forest plants (20). The common name of the species refers to its association with the pear tree. However, most of its damage is in *Acer saccharum* Marshall in the northern United States and Canada (19, 21, 22).

Taeniothrips inconsequens is native to Europe and entered America through the north of the United States and Canada (9, 15). In Argentina, it was cited on pear for the first time by Joan in 1921 (10). Then, De Santis and co-workers in 1978 (8) reported others host plants (almond, peach and apricot trees) and its geographic distribution (Buenos Aires, Mendoza and Neuquén provinces) in Argentina. In the last 20 years, the pear thrips has not been mentioned in studies involving the identification of thrips on fruit trees in Argentina (3, 4, 5, 6, 16, 23). We have never seen this thrips species in Argentina and we believe that the pear thrips is not present in this country. This study was conducted to verify the presence of *T. inconsequens* in Argentina.

Materials and methods

Sampling and collecting thrips from fruit branches

The sampling was carried out between 2017 and 2019 during the flowering or sprouting stages of nine species of fruit trees in the six main producing provinces of stone and pip fruits in Argentina (tables 1, page 111 and 2, page 112).

The sampling was performed by manually shaking a branch over a 0.40 m x 0.30 m white tray. Thrips collected on the tray were transferred with a brush to vials with preservative liquid (10% ethyl alcohol aqueous solution, 5% acetic acid and 0.1% Triton; Bhatti, J., personal communication). Collected thrips were placed in a 70% ethanol aqueous solution for their final conservation.

Table 1. Number of fruit orchards, ordered by provinces and counties, with their respective geographic coordinates, altitude ranges and sampling times.

Tabla 1. Número de huertos frutales muestreados, ordenadas por provincias y departamentos, con sus respectivas coordenadas geográficas, rangos de altitudes y épocas de muestreo.

Province	County	Geographical coordinates	Altitude (meters above sea level)	Sampling Date	Number of plots
Buenos Aires	Baradero	S33°48′ W 59°31′	25	sep-2019	2
	Luján	S34°34′ W 59°06'	31-37	oct-2019	3
	Mercedes	S34°39′ W59°26′	35-38	sep-2019	1
	San Pedro	S33°40′ W59°40′	19-38	sep-oct-2019	22
Chubut	Gaiman	S43°17′′ W65°29'	218	oct-2019	1
	Sarmiento	S45°36′ W69°05′	271-279	oct-2019	3
Mendoza	General Alvear	S34°58′ W67°42′	451-506	aug-oct-2017	49
	Junín	S33°08′ W68°28′	655-712	sep-oct-2017	13
	Lavalle	S32°43′ W68°35′	603-613	oct-nov-2017	13
	Luján de Cuyo	S33°01′ W68°52′	719-984	sep-2017	4
	Maipú	S32°58′ W68°45′	650-815	aug-oct-2017	24
	Rivadavia	S33°11′ W68°28′	658-662	aug-oct-2017	4
	San Carlos	S33°46 ′W69°02′	939-1030	oct-2017	6
	San Martín	S33°04′ W68°28′	600-658	aug-nov-2017	25
	San Rafael	S34°37′′ W68°20′	478-726	aug-nov-2017	63
	Tunuyán	S33°34′ W69°01′	944-1115	aug-oct-2017	12
	Tupungato	S33°22′ W69°08′	979-1240	sep-2017	16
Neuquén	Confluencia	S38°57′ W68°03′	293-307	sep-oct-2017	8
	Añelo	S38°21′ W68°47′	350	oct-2017	1
Río Negro	General Roca	S39°02′ W67°35′	202-309	sep-oct-2017	25
San Juan	Albardón	S31°25′ W68°30′	626-676	aug-sep-2018	29
	Angaco	S31°24′ W68°22′	590	sep-2018	2
	Calingasta	S31°22′ W69°30'	1448	sep-2018	1
	Chimbas	S31°29′ W68°32′	608	sep-2018	1
	Jáchal	S30°14′ W68°45'	795-1262	sep-2018	4
	Nueve de Julio	S31°37′ W68°23′	580	sep-2018	1
	Pocito	S31°39′ W68°33′	581-684	aug-sep-2018	29
	Rawson	S31°34′ W68°31′	596	sep-2018	1
	Rivadavia	S31°31′ W68°36′	684-705	sep-2018	4
	San Martín	S31°30′ W68°17′	588-592	aug-sep-2018	5
	Ullún	S31°25′ W68°44'	791-794	sep-2018	3
	Veinticinco de mayo	S31°49′ W68°12′	555-564	sep-2018	4
	Zonda	S31°33′ W68°46'	774-785	aug-sep-2018	8
Santa Cruz	Lago Buenos Aires	S46°35′ W70°55'	210-229	jan-feb-2019	6

Table 2. Numbers of thrips collected by species and province on a total of 393 samples taken in Argentina.

Tabla 2. Números de trips recolectados por especies y provincia sobre un total de 393 muestras tomadas en la Argentina.

		Almond	**Apple**	**Apricot**	**Cherry**	**Peach**	**Pear**	**Plum**	**Quince tree**
Buenos Aires	*F. gemina*	-	-	-	-	638	16	12	-
	F. occidentalis	-	-	-	-	1	0	0	-
	T. tabaci	-	-	-	-	22	17	3	-
	Other	-	-	-	-	46	0	0	-
	Larvae	-	-	-	-	17	0	0	-
Chubut	*F. australis*	-	-	-	13	-	-	-	-
	F. occidentalis	-	-	-	36	-	-	-	-
	T. tabaci	-	-	-	7	-	-	-	-
	Haplothrips spp.	-	-	-	4	-	-	-	-
Mendoza	*F. australis*	139	70	135	67	831	69	383	149
	F. gemina	12	0	25	0	120	43	102	41
	F. occidentalis	15	3	126	27	204	35	180	163
	T. tabaci	58	25	236	67	572	467	502	820
	Haplothrips spp.	0	1	50	1	30	8	16	17
	Other	0	0	15	4	29	43	12	12
	Larvae	3	0	50	2	79	88	51	126
Neuquén	*F. australis*	-	12	-	-	-	26	-	-
	F. occidentalis	-	34	-	-	-	44	-	-
	T. tabaci	-	3	-	-	-	10	-	-
	Haplothrips spp.	-	25	-	-	-	29	-	-
	Other	-	1	-	-	-	1	-	-
Río Negro	*F. australis*	-	10	-	-	-	24	-	-
	F. occidentalis	-	67	-	-	-	89	-	-
	T. tabaci	-	30	-	-	-	26	-	-
	Haplothrips spp.	-	120	-	-	-	118	-	-
	Other	-	2	-	-	-	2	-	-
San Juan	*F. australis*	474	-	406	-	440	-	564	115
	F. gemina	8	-	6	-	7	-	12	16
	F. occidentalis	31	-	42	-	16	-	79	47
	T. tabaci	69	-	26	-	13	-	41	44
	Haplothrips spp.	1	-	0	-	0	-	0	-
	Larvae	15	-	16	-	9	-	24	2
Santa Cruz	*F. occidentalis*	-	-	-	308	-	-	-	-
	Other	-	-	-	27	-	-	-	-
	General *Total*	825	403	1133	563	3074	1155	1981	1552

Thrips were collected using systematic U sampling. For every ten plants one was sampled. One branch at 1.5 m height per tree was selected for the sampling. Thrips were collected completing approximately a sample of 30 specimens per orchard. When the number of thrips was low, all the specimens were collected in a time period of 30 minutes.

Geographic coordinates were recorded with a G.P.S., dates and plant species and phenological stage were registered.

Thrips identification

Most of the adult specimens were identified under a stereoscopic microscope with an 80 x magnification. Some specimens of each species were mounted on microscopic slides following the Mound and Marullo technique (12) and identified by keys and descriptions (1, 4, 7, 8, 9, 14) or by confrontation with previously identified material from the thrips collections of INTA Mendoza (EEA Mza INTA) and of the Museo de Ciencias Naturales de La Plata (MLP https://www.museo.fcnym.unlp.edu.ar/). All specimens collected were preserved in vials with 70% ethanol or microscopic slides at the Entomology Lab of the EEA Mendoza INTA.

Results

A total of 10,686 individuals of thrips from 393 samples of fruit branches from the main stone and pip fruit producing areas of Argentina were collected. *Taeniothrips inconsequens* (figure 1A, page 114) was not found. Ninety percent of the collected thrips corresponded to four species: 37% *Frankliniella australis* (figure 1B, G y E, page 114), 29% *Thrips tabaci* Lindeman (figure 1C, page 114), 14% *Frankliniella occidentalis* (Pergande), and 10% *Frankliniella gemina* Bagnall. The remaining 10% consisted of 2% larvae and 8% adults of the following species, *Aneristothrips rostratus* De Santis, *Frankliniella frumenti* Moulton, *Frankliniella schultzei* (Trybom), *Frankliniella inesae* de Borbón & Zamar, *Frankliniella juancarlosi* de Borbón & Zamar, *Frankliniella* spp, *Leptothrips mali* (Fitch), *Aeolothrips fasciatipennis* Blanchard, *Arorathrips texanus* (Andre), *Tenothrips frici* (Uzel), *Haplothrips* spp, *Haplothrips fiebrigi* Priesner, *Haplothrips trellesi* Moulton, *Thrips australis* (Bagnall), *Karnyothrips* spp., *Caliothrips phaseoli* (Hood).

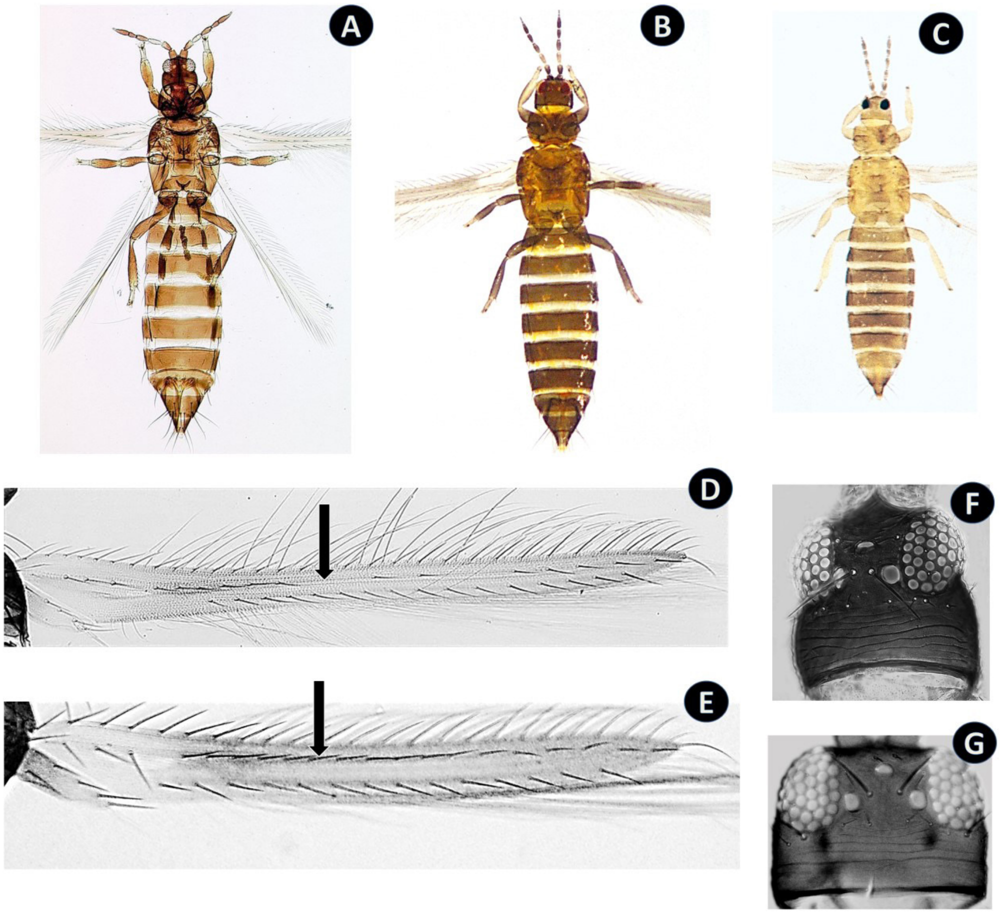
Regarding the distribution of the species in the studied areas, *F. gemina* was dominant in Buenos Aires, *T. tabaci* in Mendoza, *Haplothrips* spp. in Río Negro and Neuquén, *F. occidentalis* in Chubut and Santa Cruz, and *F. australis* Morgan in San Juan (table 2, page 112). In relation to the host plants, *F. australis* was dominant in almond and peach trees, while *T. tabaci* was dominant in pear and quince trees.

Discussion

In Joan's work (1991), samples of pear flowers were collected only in the Ituzaingó county in the province of Buenos Aires. The characters used by Joan to describe and illustrate *T. inconsequence* are shared by many other thrips species or are imprecise and diagnostic features of pear thrips are not specified. Furthermore, the author does not indicate where the examined material was deposited in order to study it. The citation about the pear thrips for the province of Buenos Aires (10) can be attributed to a misidentification. This error could be due to the limited knowledge about thrips taxonomy at that time, the limited availability of information and the non-corroboration of the species determination by a thrips specialist.

Joan (1991) describes a species of brown colour, with eight-segmented antennae, and a head that is wider than long. Although the first two features are correct, the last one is erroneous. The head of *T. inconsequens* is longer than wide (9, 14). Also in Joan's paper, the illustrations are imprecise, the larva drawing shows more information, with respiration spiracles in tergites II and VIII and numerous tooth-shaped processes in tergite IX. The distribution of the setae in the tergites and the shape of the antenna are those observed in species of the family Thripidae. If the illustration of Joan's larva is correct, the pear thrips is ruled out because the tooth-shaped processes of tergite IX are medium size and numerous, while in *T. inconsequens* they are large and scarce (three pairs) (17).

Other common species on fruit trees in Buenos Aires could also motivate erroneous identifications. Among the most common brown species that can be found is *T. tabaci*. This species differs from *T. incosequens* in that it has uniformly pale forewings and the head is wider than long, and it does not show constriction (figure 1A-G, page 114). Another frequent, brown-coloured species is *Frankliniella schultzei*. It is distinguished from *T. inconsequens* because adults have a pair of long setae on the anterior margin and another on the anterior angles of the pronotum. The forewings are uniformly pale, the head is wider than long and without posterior constriction to the eyes.



Adult female. A, *T. inconsequens* (image by Mound *et al.*, 2018); B, *F. australis;* C, *T. tabaci.* Fore wing. D, *T. inconsequens* (image by Mound *et al.*, 2018); E, *F. australis* (indicated with an arrow the first vein of the fore wing). Head, F, *T. inconsequens*; G, *F. australis.*

Hembras adultas. A, *T. inconsequens* (imagen de Mound *et al.*, 2018); B, *F. australis*; C, *T. tabaci*. Alas anteriores. D, *T. inconsequens* (imagen de Mound *et al.*, 2018); E, *F. australis* (indicado con una flecha la primera vena del ala anterior). Cabezas, F, *T. inconsequens*; G, *F. australis.*

Figure 1. Comparison between *Taeniothrips inconsequens, Frankliniella australis* and, *Thrips tabaci.*

Figura 1. Comparación de *Taeniothrips inconsequens* con *Frankliniella australis* y *Thrips tabaci.*

Pear thrips is also cited by De Santis and co-workers (1978). These authors provide data on the host species (pear, almond, peach, and apricot) and their geographic distribution for Argentina (Buenos Aires, Mendoza and Río Negro). However, they do not indicate where this information was obtained from. On the other hand, no slides labelled as *T. inconsequens* with specimens collected in Argentina are at the Museo de la Plata.

The citations of *T. inconsequens* in Mendoza and Río Negro (2, 8) can be attributed to mistakes over mistakes which originated in erroneous identifications made by non-specialists and later mentioned in successive citations. *Frankliniella australis* (figure 1B) resembles *T. inconsequens* (figure 1A) in its body and antenna coloration. To distinguish them, it is necessary to observe in detail the pronotum setae, the venation of the forewings and the shape of the head, characteristics that can be visualized under a stereomicroscope at 80 x magnification. *Taeniothrips inconsequens* does not have long setae on the margin and anterior angles of the pronotum, it has the first vein with setae arranged discontinuously (figure 1D) and the head has a constriction posterior to the eyes giving the appearance of swollen genae; (figure 1F) while *F. australis*, (figure 1E) has a pair of long setae in the anterior margin and another in the anterior angles of the pronotum, the first vein has a continuous row of setae; the margins of the genae are almost parallel, with no obvious constriction behind the eyes (figure 1G). These species also have other important differences. *T. inconsequens* has only two pairs of ocellar setae and has a small spur on the tarsal apex of the forelegs (a character that differentiates it from other species of the genus *Taeniothrips*), and it does not have ctenidia placed anterior to the spiracles of respiration. While *F. australis* has three pairs of ocellar setae, a spur on the tarsi of the fore legs is not present, and tergite VIII has a pair of ctenidia located anterior to the spiracles of respiration.

Considering that no slides of specimens collected in Argentina of *T. inconsequens* were found at the Museo de La Plata, in De Santis and co-workers' paper (1978), it is considered that these authors make references to other articles that cite the species but no to identifications made by themselves.

In our research, the presence of some species of thrips in fruit trees was accidental due to the fact that they have other host plants. Thus, *Arorathrips texanus* and *Frankliniella frumenti* live on grasses, while *Tenothrips frici* is common on asteraceae, *Thrips australis* on eucalyptus, *Frankliniella inesae* on asteraceae, mainly of the *Baccharis* genus. Some species found in low frequency, which are predators or potential predators, were *Leptothrips mali*, *Aeolothrips faciatipennis* and *Karnyothrips* spp. Other species, such as *F. schultzei* and *Caliothrips phaseoli*, may have alternative fruit hosts.

Conclusions

It is possible, based on our findings, that the citation of *T. inconsequens* for Argentina is a misidentification of another species. The pear thrips was not found during blooming and or sprouting of nine kinds of stone and pip fruit in Argentina's primary producing regions. In addition, no slides of *T. inconsequens* from Argentina were found at the museum of La Plata. The pear thrips should be excluded from the Argentine fauna and considered as a quarantine species to prevent its presence.

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Competitive ability of bean *Phaseolus vulgaris* cultivars with *Urochloa plantaginea*

Capacidad competitiva de cultivares de frijol *Phaseolus vulgaris* con *Urochloa plantaginea*

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Abstract

The objective of this work was to evaluate the competitive ability of carioca bean cultivars BRS FC104, BRS FC402, IAC Imperador, IAC Milênio, IPR Tangará, and SCS Riqueza in the presence of Alexandergrass (*Urochloa plantaginea*) in different proportions of plants in association. The experiments were carried out in a greenhouse in a completely randomized design with four replications. The treatments were arranged according to the proportions of beans and Alexandergrass plants: 100:0, 75:25, 50:50, 25:75, and 0:100 or 40:00, 30:10, 20:20, 10:30, and 0:40 plants per pot. The competitiveness of the species was analyzed using diagrams applied to replacement experiments and also through relative competitiveness indices. At 30 days after crop emergence, leaf area (LA), height (HP), diameter (DP), and shoot dry mass (SM) of the plant shoots were measured, as well as variables related to the physiology of the species. There was competition among carioca bean cultivars in the presence of Alexandergrass regardless of the proportion of plants, causing decreases in DP, LA and SM. Basically, there is competition for the same environmental resources between beans and Alexandergrass.

Keywords
Phaseolus vulgaris • weed ecophysiology • competitive interaction • replacement series

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Resumen

El objetivo de este trabajo fue evaluar la capacidad competitiva de cultivares de frijol tipo carioca, BRS FC104, BRS FC402, IAC Imperador, IAC Milênio, IPR Tangará y SCS Riqueza, bajo niveles crecientes de infestación de Alexandergrass (*Urochloa plantaginea*). Los experimentos se realizaron en condiciones de invernadero en un diseño completamente al azar con cuatro repeticiones. Los tratamientos se organizaron en proporciones de plantas de frijol y Alexandergrass de: 100: 0, 75:25, 50:50, 25:75 y 0: 100 o 40:00, 30:10, 20:20, 10:30 y 0: 40 plantas por maceta. El análisis de la competitividad de las especies se realizó mediante diagramas aplicados a experimentos de series de reemplazo y también mediante los índices de competitividad relativa. Treinta días después de la emergencia del cultivo, se midieron el área foliar (AF), la altura de la planta (AP), el diámetro de la planta (DP) y la masa seca de los brotes (MS), además de características relacionadas con la fisiología de la planta. Hubo competencia entre cultivares de frijol carioca en presencia de Alexandergrass, independientemente de la proporción de plantas, lo que provocó reducciones en el DP, AF, y MS. Básicamente, existe competencia por los mismos recursos ambientales entre el cultivo de frijoles y Alexandergrass.

Palabras clave

Phaseolus vulgaris • ecofisiología de malezas • interacción • serie de reemplazo

Introduction

Brazilian population has a habit of consuming beans (*Phaseolus vulgaris* L.), which is produced in all regions of the country and destined almost entirely to domestic consumption. The crop has a short cycle, which represents a great advantage for farmers, as it is possible to sow two crops (corn or soybeans) followed by beans in the same area and agricultural year. Thus, in Brazil, beans can be sown in three different seasons, known as the water, drought, and winter seasons, thus providing a constant supply to the domestic market throughout the year (4).

The average production of Brazilian bean grains in the 2020/21 harvest was 1.1 t ha^{-1} (4). Among the factors that may explain this low productivity, one is the negative interference of weeds (24), which can cause losses in bean grain yields that can exceed 82% when control measures are not adopted (11, 17, 23).

Weeds reduce bean grain yields due to competition for environmental resources, such as nutrients, light, and water; in addition, they can release allelopathic compounds to the soil, resulting in qualitative and quantitative losses. They can also be responsible for indirect damage,hosting diseases and insects (10, 11, 16, 21). Weeds at the end of the cycle can also interfere with the quality by depreciating the harvested product and making it difficult to harvest, either manual or mechanical (11, 14, 24).

Among the weeds infesting bean fields, it is worth mentioning that the Alexandergrass (*Urochloa plantaginea* (Link) R.D. Webster.) is one of the main weeds in the South, Southeast, and Midwest regions of Brazil, and in many cases, it is responsible for a high degree of damage (14). Alexandergrass is a C4 annual grass reproduced by seeds and is one of the main weeds competing directly for environmental resources (24). These plants grow quickly and infest large tracts of land easily and quickly, consequently shading the crops (10, 13, 14).

Weed management in bean fields is performed through chemical control in most cases due to its efficiency and lower cost when compared to other control methods. However, when used continuously, it can cause problems to the environment and to the applicator, leaving residues in grains and, in several cases, causing phytotoxicity to the crop (3). Thus, there is an increasing demand for more sustainable production models. For the development of weed control strategies in agricultural crops, it is necessary to know their traits.

The determination of competitive interactions between crops and weeds requires experimental designs and appropriate analysis methods. One of the most used is the substitutive series, where different densities of weed and crop plants are used (1, 10, 12, 14). Because weed density is variable according to environmental conditions and the initial soil seed bank, the degree of infestation can be changed (2). The greater competitiveness of one species in relation to another indicates that it will have a greater capacity to assimilate resources and, therefore, have a greater potential to grow and develop (1, 2, 12). Choosing more competitive cultivars gives an advantage to the crop over the weeds. The rapid initial growth of crop plants supresses weed growth (2, 9).

Thus, the development of strategies that will minimize the effects of weed competitiveness with crops, for example, most competitive bean cultivars, will greatly contribute to superior grain yields. Therefore, the objective of this work was to evaluate the competitive ability of carioca type bean cultivars BRS FC104, BRS FC402, IAC Imperador, IAC Milênio, IPR Tangará, and SCS Riqueza in the presence of Alexandergrass as a function of plant proportion.

Material and methods

The experiments were conducted in a greenhouse at the Federal University of Fronteira Sul (UFFS), Campus Erechim, RS, Brazil, in the 2019/20 cropping season. Experimental units consisted of plastic pots with a capacity of 8 dm^3 filled with soil from an arable area, characterized as humic Oxisol (22). Correction of pH and soil fertilization were carried out according to soil analysis and following the technical recommendations for common beans (19). The chemical and physical soil characteristics were: pH_{water}= 4.8; organic matter (OM)= 3.5%; P= 4.0 mg dm^{-3}; K= 117.0 mg dm^{-3}; Al^3+ = 0.6 $cmol_c$ dm^{-3}; Ca^2+= 4.7 $cmol_c$ dm^{-3}; Mg^2+ = 1.8 $cmol_c$ dm^{-3}; effective cation exchange capacity (CEC)= 7.4 $cmol_c$ dm^{-3}; CEC at pH_7= 16.5 $cmol_c$ dm^{-3}; H + Al = 9.7 cmolc dm^{-3}; sum of bases (SB)= 6.8 $cmol_c$ dm^{-3}; aluminum saturation (V)= 41% and Clay= 60%.

The experimental design was completely randomized with four replications. Tested competitors included the bean (*Phaseolus vulgaris*) cultivars BRS FC104, BRS FC402, IAC Imperador, IAC Milênio, IPR Tangará, and SCS Riqueza Wealth, which competed with Alexandergrass (*Urochloa plantaginea*). Preliminary experiments were carried out for both beans and Alexandergrass growing single, with the objective of determining the density of plants in which the final production becomes constant. For this, populations of 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, and 64 plants per pot were used (equivalent to 25, 49, 98, 196, 392, 587, 784, 980, 1,176, 1,372, and 1,568 plants m^{-2}). Twenty five days after emergence, the aboveground masses of beans and Alexandergrass were collected to determine the aboveground dry mass (DM), which was quantified by weighing after being dried in a forced air circulation at a temperature of 60 ± 5°C until reaching constant mass. Through the average DM values of the species, the constant DM production was obtained with densities of 40 plants per pot for Alexandergrass and all bean cultivars, which was equivalent to 650 plants m^{-2} (data not shown).

After the identification of the constant final density, six other experiments were installed to evaluate the competitiveness of the bean cultivars BRS FC104, BRS FC402, IAC Imperador, IAC Milênio, IPR Tangará, and SCS Riqueza with Alexandergrass. All experimens were conducted in substitutive series in different combinations of cultivars and weeds, varying the relative proportions of plants per pot (40:0; 30:10; 20:20; 10:30, and 0:40) while maintaining a constant total plant density (40 plants per pot). In order to establish the desired densities in each treatment and to obtain uniform seedlings, the seeds were previously sown in trays and later transplanted to the pots.

Thirty five days after the emergency (DAE), plant height (EP), stem diameter (DP), leaf area (AF), and aboveground dry mass (DM) were measured. The plant height (cm) was determined with a ruler from the soil surface to the apex meristem of the plants. The stem diameter (mm) was measured with the aid of a digital caliper, 5 cm above soil surface. For the determination of the AF, a portable leaf area meter model CI-203 (BioScience Inc.) was used. After determining the AF, the plants were packed in paper bags and put into an oven for dry mass determination.

Thirty DAE, the plant physiology of the bean cultivars was measured. The CO_2 concentration in the substomatal chamber (*Ci* - µmol mol^{1}), photosynthetic rate (*A* - µmol m^{2} s^{1}), stomatal conductance (*Gs* - mol m^{2} s^{1}) and transpiration rate (*E* - mol_{H2O} m^{2} s^{1}), carboxylation efficiency (*EC* - mol_{CO2} m^{2} s^{1}), and water use efficiency (*WUE* - mol_{CO2} mol_{H2O}^{-1}) were assessed.

These variables were determined in the canopy of the bean plants in the first fully expanded leaf. To assess the physiological variables, an infrared gas analyzer (IRGA) ADC LCA PRO (Analytical Development Co. Ltd, Hoddesdon, UK) was used between 8 and 11 hours in the morning. One experiment was assessed per day.

Data were analyzed by the method of graphical analysis of variation or relative productivity (1, 2, 5). This procedure, also known as the conventional method for substitutive experiments, consists of the construction of diagrams based on the relative (PR) or total (PRT) productivities. When PR is a straight line, it means that the ability of the species is equivalent. If PR results in a concave line, it indicates that there is a loss in the growth of one or both species. On the contrary, if the PR shows a convex line, there is a benefit in the growth of one or both species. When PRT is equal to 1 (straight line), competition for the same resources occurs; if it is greater than 1 (convex line), competition is avoided. If PRT is less than 1 (concave line), there is mutual harm in growth (5). The relative competitiveness indexes (CR), relative clustering coefficient (K), and aggressiveness (A) of the species were also calculated. CR represents the comparative growth of common bean cultivars (X) in relation to the competitor Alexandergrass (Y). K indicates the relative dominance of one species over the other, and A indicates which species is more aggressive. Thus, CR, K, and A indicate which species is most competitive, and their joint interpretation determines with greater certainty the competitiveness of the species (5). Common bean cultivars X are more competitive than Alexandergrass Y when CR > 1, Kx > Ky, and A > 0. On the other hand, Alexandergrass Y is more competitive than bean cultivars X when CR < 1, Kx < Ky, and A < 0 (2). To calculate these indices, the 50:50 proportions of the species involved in the experiments were used, using the equations: $CR = {}^{Prx}/_{Pry}$; $Kx = {}^{Prx}/_{(1\ Prx)}$; $Ky = {}^{Pry}/_{(1-Pry)}$; $A = Prx\ PRy$, according to Cousens and O'Neill (1993).

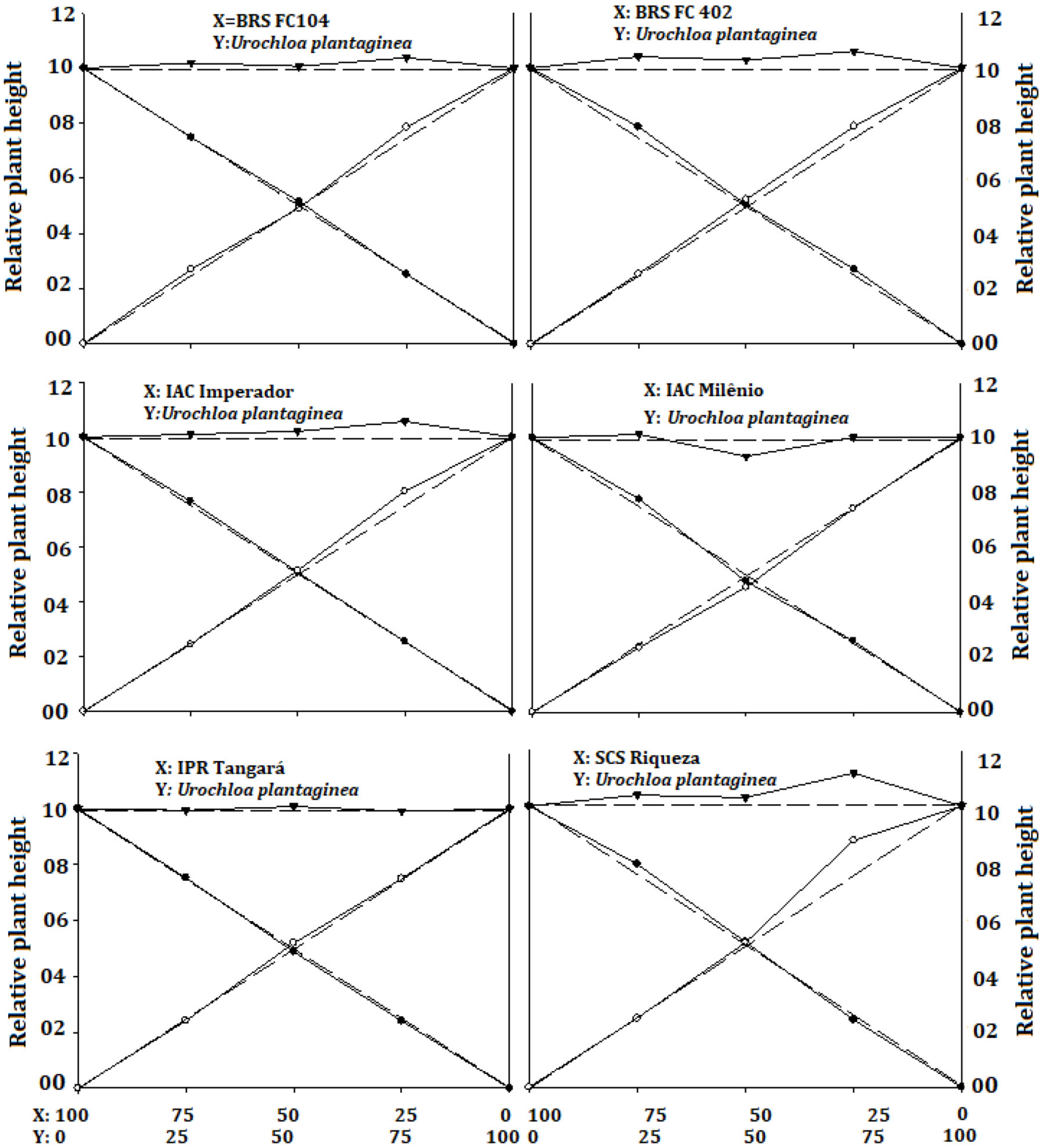
The procedure of the statistical analysis of productivity or relative variation included the calculation of the differences for the PR (DPR) values, obtained in the proportions 25, 50, and 75% in relation to the values belonging to the hypothetical line in the respective proportions (2, 5). The t-test was used to test the differences related to DPR, PRT, CR, K, and A (2, 5). To test the differences of DPR and A, it was considered a null hypothesis when the averages are equal to zero (Ho = 0). For PRT and CR, the null hypothesis is when the averages are equal to one (Ho = 1), and for K, the null hypothesis is if the averages of the differences between Kx and Ky are equal to zero [Ho = (Kx Ky) = 0]. The criterion for considering the PR and PRT curves to be different from the hypothetical lines was that, at least in two proportions, significant differences occurred by the t-test (2). Likewise, for CR, K, and A, differences in the competitiveness were considered when there was a significant difference by the t-test in at least two of them.

The results obtained for EP, DC, AF, MS, and the physiological variables, expressed as mean values per treatment, were subjected to analysis of variance by the F-test; when significant, the means were compared by Dunnett's test. Monocultures were considered the control in these comparisons. In all the statistical analyses, a significance of $p \leq 0.05$ was adopted. All graphics and coefficients were obtained using the statistical environment "R" (18).

Results and discussion

All carioca bean cultivars showed similarities regarding the competition. They were all harmed by Alexandergrass with significant differences for the height, diameter, dry weight, and leaf area for all plant proportions (figure 1, page 121; figure 2, page 122; figure 3, page 123, figure 4, page 124). With regard to PRT, there were no statistical differences between the expected and estimated values only for the plant height, where it presented average values close to 1 in all combinations (figure 1, page 121; table 1, page 125), demonstrating that the bean and Alexandergrass compete for the same environmental resources.

For the plant diameter (figure 2, page 122), the expected and estimated values of PRT were higher and lower than 1, respectively, and for leaf area and aboveground dry mass (figures 3 , page 123 and figure 4, page 124), the values were all less than 1. When the PRT has concave lines and values less than 1, it is possible to infer that there was competition between crop and weed for the same environmental resources. Rubin *et al.* (2014) and Galon *et al.* (2017) reported that when PRT is less than 1, there is mutual antagonism.

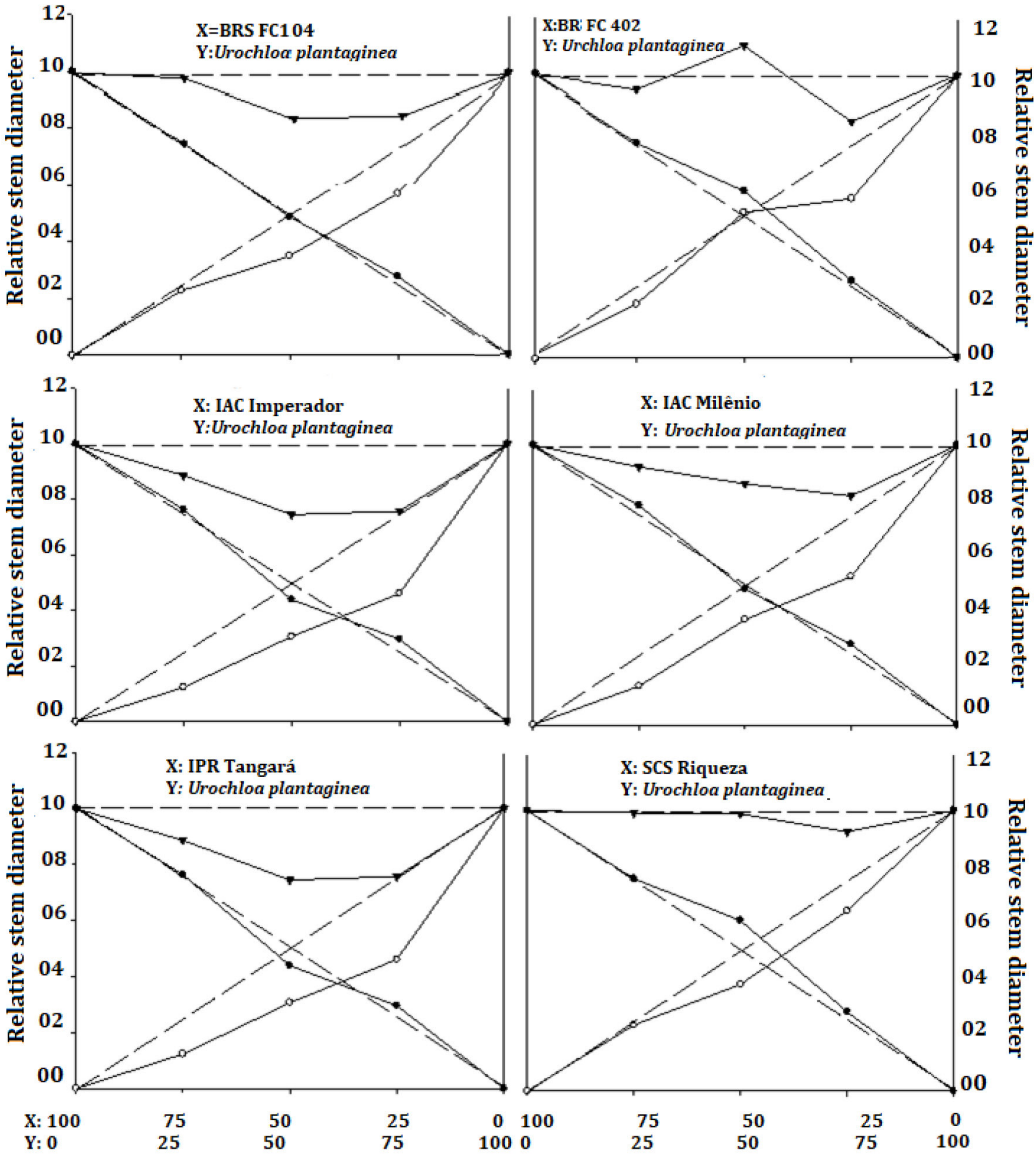


Dashed lines represent the expected values; solid lines represent the observed values.

Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados.

Figure 1. Relative productivity (PR) for plant height of beans (●) and *Urochloa plantaginea* (o), and total relative productivity (PRT) of the plant community (▾) as a function of plant proportion.

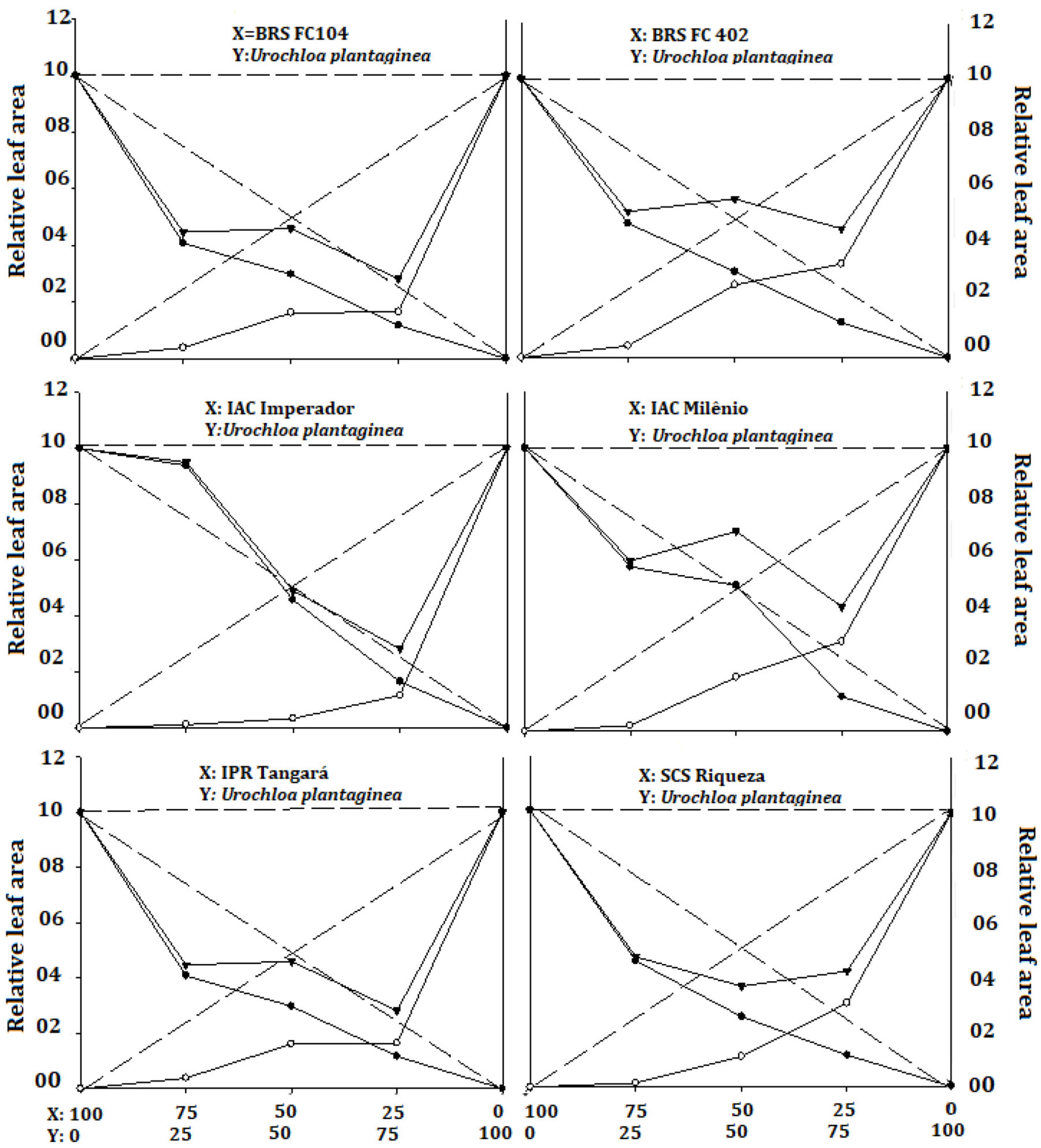
Figura 1. Productividad relativa (PR) para la altura de planta de frijol (●) y *Urochloa plantaginea* (o), y productividad relativa total (PRT) de la comunidad de plantas (▾) en función de la proporción de plantas.



Dashed lines represent the expected values; solid lines represent the observed values.
Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados.

Figure 2. Relative productivity (PR) for stem diameter of beans (●) and *Urochloa plantaginea* (o), and total relative productivity (PRT) of the plant community (▾) as a function of plant proportion.

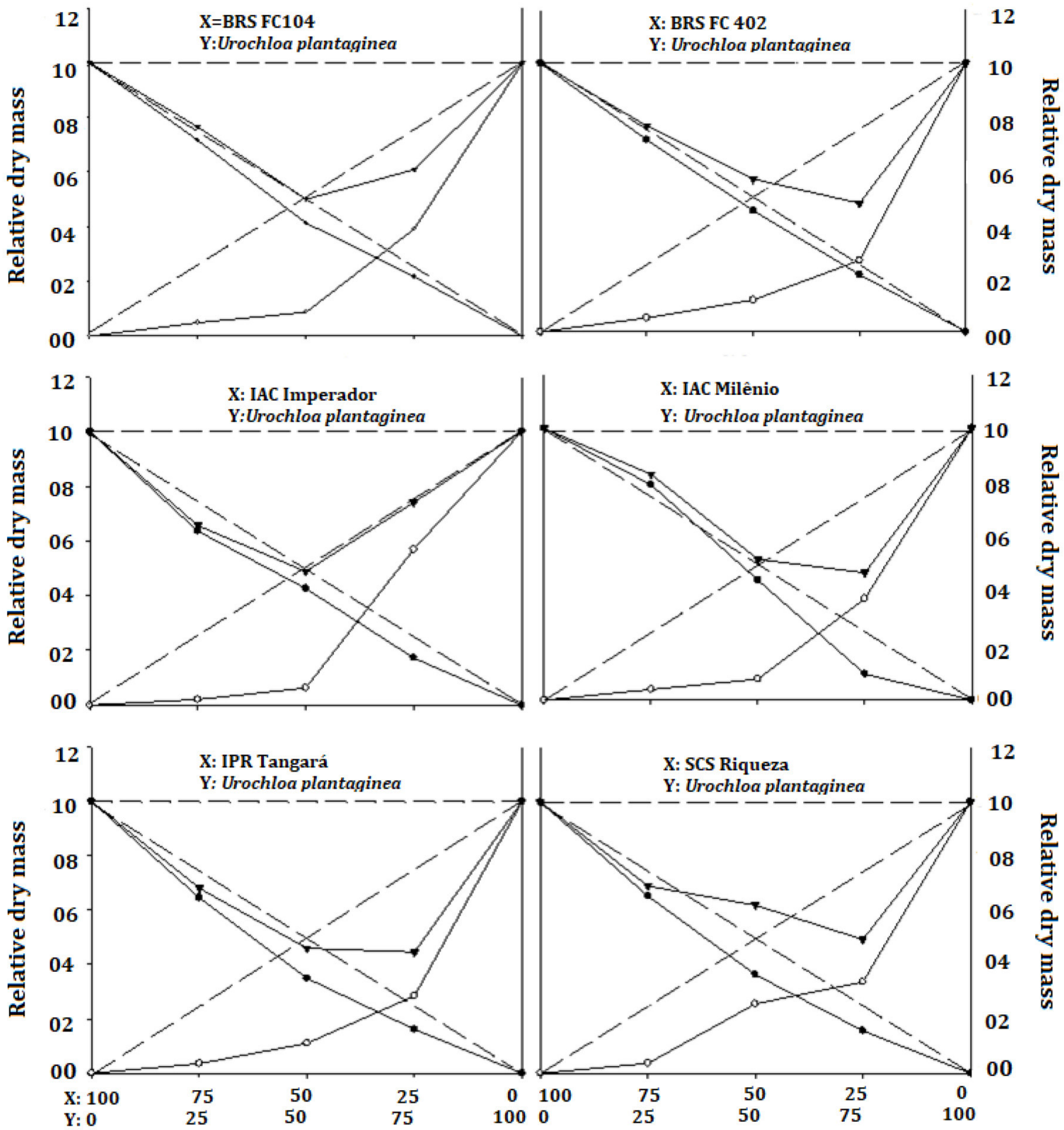
Figura 2. Productividad relativa (PR) para el diámetro del tallo de frijol (●) y *Urochloa plantaginea* (o), y productividad relativa total (PRT) de la comunidad vegetal (▾) en función de la proporción de plantas.



Dashed lines represent the expected values; solid lines represent the observed values.
Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados.

Figura 3. Relative productivity (PR) for leaf area of beans (●) and *Urochloa plantaginea* (o), and total relative productivity (PRT) of the plant community (▾) as a function of plant proportion.

Figura 3. Productividad relativa (PR) para el área foliar de frijol (●) y *Urochloa plantaginea* (o), y productividad relativa total (PRT) de la comunidad vegetal (▾) en función de la proporción de plantas.



Dashed lines represent the expected values; solid lines represent the observed values.
Las líneas discontinuas representan los valores esperados; las líneas continuas representan los valores observados.

Figura 4. Relative productivity (PR) for shoot dry mass of beans (●) and *Urochloa plantaginea* (o), and total relative productivity (PRT) of the plant community (▾) as a function of plant proportion.

Figura 4. Productividad relativa (PR) para la masa seca aérea de frijol (●) y *Urochloa plantaginea* (o), y productividad relativa total (PRT) de la comunidad vegetal (▾) en función de la proporción de plantas.

Table 1. Relative differences for carioca bean cultivars against the competitor *Urochloa plantaginea*, available 30 days after crop emergence. UFFS, Campus Erechim, RS, 2019/2020.

Tabla 1. Diferencias relativas para los cultivares de frijol tipo carioca frente al competidor *Urochloa plantaginea*, disponibles a los 30 días después de la emergencia del cultivo. UFFS, Campus Erechim, RS, 2019/2020.

Cultivar	Plant proportion (bean: Alexandergrass)		
	75:25	50:50	25:75
Plant height			
BRS FC 104	-0.001 (±0.03)	0.02 (±0.02)	0.002 (±0.02)
BRS FC 402	0.04 (±0.02)	0.004 (±0.02)	0.02 (±0.01)*
IAC Imperador	0.02 (±0.02)	0.01 (±0.01)	0.004 (±0.01)
IAC Milênio	0.03 (±0.02)	-0.02 (±0.02)	0.01 (±0.04)
IPR Tangará	0.003 (±0.02)	-0.01 (±0.01)	-0.01 (±0.001)
SCS 205 Riqueza	0.04 (±0.01)*	0.02 (±0.02)	-0.01 (±0.01)
Stem diameter			
BRS FC 104	0.002 (±0.05)	-0.01 (±0.03)	0.03 (±0.03)
BRS FC 402	0.005 (±0.03)	0.09 (±0.04)	0.02 (±0.03)
IAC Imperador	0.01 (±0.04)	-0.06 (±0.01)*	0.05 (±0.01)*
IAC Milênio	0.03 (±0.07)	-0.01 (±0.03)	0.04 (±0.01)*
IPR Tangará	-0.07 (±0.07)	0.02 (±0.03)	-0.02 (±0.02)
SCS 205 Riqueza	0.01 (±0.01)	0.11 (±0.02)*	0.03 (±0.02)
Leaf area			
BRS FC 104	-0.22 (±0.15)	-0.24 (±0.05)*	-0.12 (±0.04)
BRS FC 402	-0.27 (±0.14)	-0.19 (±0.02)*	-0.12 (±0.04)*
IAC Imperador	0.19 (±0.16)	-0.04 (±0.02)	-0.08 (±0.03)
IAC Milênio	-0.17 (±0.16)	0.01 (±0.09)	-0.13 (±0.02)*
IPR Tangará	-0.21 (±0.05)*	-0.20 (±0.06)*	-0.13 (±0.03)*
SCS 205 Riqueza	-0.29 (±0.09)*	-0.25 (±0.09)	-0.13 (±0.04)*
Aboveground dry mass			
BRS FC 104	-0.03 (±0.07)	-0.09 (±0.03)	-0.03 (±0.01)*
BRS FC 402	-0.03 (±0.04)	-0.05 (±0.02)	-0.04 (±0.03)
IAC Imperador	-0.11 (±0.13)	-0.07 (±0.02)*	-0.08 (±0.0001)*
IAC Milênio	0.04 (±0.04)	-0.06 (±0.02)	-0.15 (±0.02)*
IPR Tangará	-0.10 (±0.06)	-0.15 (±0.04)*	-0.09 (±0.01)*
SCS 205 Riqueza	-0.10 (±0.04)	-0.14 (±0.03)*	-0.09 (±0.01)*

* Significant difference against the competitor, according to the t test ($p \leq 0.05$). Values into brackets represent the mean standard error. * Diferencia significativa frente al competidor, según la prueba t ($p \leq 0,05$). Los valores entre paréntesis representan el error estándar medio.

The stem diameter of BRS FC 104, BRS FC 402, IAC Imperador, IAC Milênio, IPR Tangará, and SCS 205 Riqueza showed deviations from the PR lines in relation to the expected lines, indicating that the crop was more damaged than the weed (figure 2, page 122).

The PRT values were less than 1 for BRS FC 104, IAC Imperador, IAC Milenio, and IPR Tangará, demonstrating damage to both crop and weed, whereas BRS FC 402 and SCS 205 Riqueza showed deviations in only one point. In order to be considered significant, they must differ in at least two plant proportions (2).

The decreases in stem diameter occurred when the crop and weed were in the proportion of 50:50; BRS FC 104, IAC Imperador, and IAC Milênio showed a decrease in the stem diameter of 2.09, 12.29, and 2.92%, respectively (figure 2, page 122; table 2, page 126). BRS FC 402, IPR Tangará, and SCS 205 Riqueza had an increase in diameter of 17.49, 4.05, and 21.56%, respectively.

The leaf area of cultivars BRS FC 104, BRS FC 402, IAC Imperador, IAC Milênio, IPR Tangará, and SCS 205 Riqueza (figure 3, page 123; table 1, page 125) showed deviations from the PR lines in relation to the expected lines, indicating that the crop was more damaged than the weed.

The PRT values (figure 3, page 123) were all lower than 1 with concave lines, indicating that both the crop and the weed were harmed. When studying the effect of weeds on soybean (9) and black beans (12), these studies reported that there were concave lines for the crop and competitor for the leaf area and dry mass, which corroborates our findings. These same authors, assessing the competitive ability of transgenic soybean cultivars with weeds, also reported similar findings for both the leaf area and dry mass. The leaf area showed decreases of up to 48.67% when the crop competed in the proportion of 50:50 with the weed (table 2), demonstrating that the crop and weed compete for the same environmental resources. Forte *et al.* (2017) also found similar results.

Table 2. Differences between carioca bean cultivars and the competitor *Urochloa plantaginea* available 30 days after emergence. UFFS, Campus Erechim-RS, 2019/20.

Tabla 2. Diferencias entre cultivares de frijol tipo carioca y el competidor *Urochloa plantaginea* disponibles a los 30 días después de la emergencia. UFFS, Campus Erechim-RS, 2019/20.

Bean:Competitor	Plant height (cm)	Stem diameter (mm)	Leaf area (cm^2 pot^{-1})	Dry mass (g pot^{-1})
BRS FC 104				
75:25	42.42	4.31	5367.76	40.92
50:50	43.83	4.21	3860.25	35.45
25:75	42.83	4.80	3951.72	37.15
BRS FC 402				
75:25	42.84	3.86	3380.82	43.55
50:50	41.17	4.50	3245.27	41.22
25:75	44.34	4.15	2658.65	38.88
IAC Imperador				
75:25	44.00	4.80	6481.76	42.29
50:50	43.67	4.14	4740.44	42.51
25:75	43.84	5.59*	3435.05	34.39
IAC Milênio				
75:25	39.50	5.36	3191.96	44.03
50:50	36.33	4.98	4245.09	36.75
25:75	39.67	5.91	2029.65	15.91*
IPR Tangará				
75:25	42.59	4.67	6086.66	43.81
50:50	41.42	5.39	4991.64*	35.57*
25:75	40.92	4.69	3925.05*	32.86*
SCS 205 Riqueza				
75:25	44.25	4.26	3889.56	47.54
50:50	43.17	5.13*	3253.80	39.66*
25:75	40.17	4.77	2953.73*	34.24*

* Mean differ from the respective control treatment (100:0 bean:Alexandergrass) according to Dunnett's ($p \leq 0.05$). * La media difiere del tratamiento de control respectivo (100: 0 frijol: Alexandergrass) según Dunnett ($p \leq 0,05$).

In the proportion 50:50 (table 2, page 126), there were decreases of 17.30, 9.74, 14.68, 11.80, 30.15, and 27.32% in the dry mass of BRS FC 104, BRS FC 402, IAC Imperador, IAC Milênio, IAC Tangará, and SCS Riqueza, respectively. Franceschetti *et al.* (2019) reported that for the black beans cultivar IPR Uirapuru, when Alexandergrass is not controlled, dry mass losses may be of the order of 48.79%.

The dry mass was reduced most significantly when the competitor was present in greater proportions than the crop. Passini *et al.* (2002) reported that 25 plants m^{-2} of Alexandergrass may cause up to 70% grain yield losses in beans. This crop, when in an equidistant plant arrangement, is most prone to suppress the weed population as well as to increase productivity (7, 13). Parreita *et al.* (2014) reported that, depending on the species, one may be more competitive than the other; however, weeds usually have a competitive advantage on resource acquisition (1, 2, 11).

The relative dominance of beans over weeds, expressed by the K indexes (K bean > K weed), shows that the crop is more competitive than Alexandergrass (table 3, page 128), which is also reflected in the aggressiveness index (A). In all comparisons, significant differences can be seen in at least two indices between bean cultivars and the competitor, showing that the crop is more competitive than the weed.

It was found that there were differences in relation to CR, K, and A when beans coexisted with Alexandergrass. Only in one situation did the weed show a higher CR, K, and A than the crop, and only for stem diameter (table 3, page 128). When placed in competition with wild genotypes, domesticated species tend to have a higher relative productivity (12, 20).

There were increases or decreases in plant physiology as a function of plant proportion. These factors may be related to the bean canopy architecture, as it can interfere with the distribution of light as well as air circulation, thus affecting CO_2 transfer and evapotranspiration. According to Ferreira *et al.* (2015), the increase in plant density causes a scarcity of resources. In other words, the yield becomes independent of the plant density after a certain level of infestation.

Bean cultivars increased the internal CO_2 concentration (Ci) when plant density increased (table 4, page 129). Ci is considered a physiological variable influenced by environmental factors such as water availability, light, and energy, among others (8). The increase in Ci for the crop may indicate an attempt to escape the stress generated by the competition but leads to remarkable harm for the physiological status of the bean plants.

The photosynthetic activity (A) as well as stomatal conductance (Gs) varied with the cultivar (table 4, page 129), showing remarkable differences among them in competition with the weed. Nascimento *et al.* (2011) reported that, as a way to avoid water stress, plants tend to close the stomata, leading to a decrease in gas exchange and, consequently, in the photosynthetic rate.

Santos *et al.* (2018b) reported that the photosynthetic rate is directly related to photosynthetically active radiation as well as to water availability and is highly dependent on stomatal opening; thus, it is a good indicator of the response of the crop to competition with weeds. The transpiration rate (E) and carboxylation efficiency (CE) varied according to the cultivar and plant proportion. Santos *et al.* (2018b) also stated that stomatal closure causes a drop in transpiration, and variations in stomata opening cause changes in water potential, as they act on the transpiration rate.

Santos *et al.* (2008b) reported that transpiration is a primary determinant of the energy balance and water status of the plant; it is mainly determined by Gs, radiation, and the deficit of atmospheric saturation, where all these variables together determine the photosynthetic capacity of the plants.

The water use efficiency (WUE) also presented different values according to the cultivar (table 4, page 129). There was a considerable decrease in the dry mass, on the other hand, for all bean cultivars, and these values are related to other physiological variables because all variables are closely linked. According to Ferreira *et al.* (2015), soybean competition with Urochloa brizantha and Bidens pilosa caused a decrease in the photosynthetic rate, transpiration, stomatal conductance, efficiency in the use of water, and aboveground dry mass of soybean, with *U. brizantha* being the most harmful to the crop.

Table 3. Competitiveness indexes between carioca bean cultivars and the competitor *Urochloa plantaginea*, expressed as relative competitiveness (CR), relative clustering coefficients (K) and aggressivity (A), from replacement series experiments. UFFS, Campus Erechim-RS, 2019/2020.

Table 3. Índices de competitividad entre cultivares de frijol tipo carioca y el competidor (*Urochloa plantaginea*), expresados como competitividad relativa (CR), coeficientes de agrupamiento relativo (K) y agresividad (A), a partir de experimentos de series sustitutivas. UFFS, Campus Erechim-RS, 2019/2020.

Cultivar	CR	Kx (bean)	A
Plant height			
BRS FC 104	1.05 (±0.06)	1.07 (±0.08)	0.02 (±0.03)
BRS FC 402	0.96 (±0.03)	1.02 (±0.06)	-0.02 (±0.02)
IAC Imperador	0.99 (±0.04)	1.03 (±0.05)	-0.01 (±0.02)
IAC Milênio	1.12 (±0.10)	0.92 (±0.07)	0.04 (±0.03)
IPR Tangará	0.94 (±0.04)	0.96 (±0.04)	-0.03 (±0.02)
SCS 205 Riqueza	1.02 (±0.09)	1.07 (±0.07)	0.002 (±0.04)
Stem diameter			
BRS FC 104	1.45 (±0.15)	0.98 (±0.10)*	0.14 (±0.04)*
BRS FC 402	1.20 (±0.14)	1.48 (±0.22)	0.08 (±0.07)
IAC Imperador	1.64 (±0.37)*	0.78 (±0.02)	0.13 (±0.06)
IAC Milênio	1.32 (±0.10)*	0.96 (±0.11)	0.11 (±0.02)*
IPR Tangará	1.65 (±0.35)	1.10 (±0.12)	0.15 (±0.07)
SCS 205 Riqueza	1.63 (±0.15)*	1.57 (±0.12)*	0.23 (±0.04)*
Leaf area			
BRS FC 104	6.12 (±2.89)	0.36 (±0.09)*	0.18 (±0.07)
BRS FC 402	2.65 (±1.58)	0.45 (±0.05)	0.05 (±0.12)
IAC Imperador	14.46 (±2.25)*	0.85 (±0.07)*	0.42 (±0.02)*
IAC Milênio	5.19 (±2.04)	1.29 (±0.44)	0.32 (±0.10)*
IPR Tangará	3.05 (±1.16)	0.46 (±0.13)	0.14 (±0.09)
SCS 205 Riqueza	8.49 (±5.48)	0.40 (±0.16)	0.14 (±0.10)
Dry mass			
BRS FC 104	4.94 (±0.54)*	0.72 (±0.11)*	0.33 (±0.03)*
BRS FC 402	3.87 (±0.21)*	0.83 (±0.07)*	0.33 (±0.02)*
IAC Imperador	7.39 (±1.29)*	0.75 (±0.07)*	0.36 (±0.02)*
IAC Milênio	6.20 (±1.11)*	0.80 (±0.07)*	0.36 (±0.03)*
IPR Tangará	3.20 (±0.33)*	0.56 (±0.10)*	0.24 (±0.04)*
SCS 205 Riqueza	1.69 (±0.35)	0.58 (±0.09)	0.11 (±0.07)

* Significant difference against the competitor, according to the t test ($p \leq 0.05$). Values into brackets represent the mean standard error. * Diferencia significativa frente al competidor, según la prueba t ($p \leq 0,05$). Los valores entre paréntesis representan el error estándar medio.

Table 4. Physiological parameters of carioca bean cultivars against the competitor *Urochloa plantaginea*, 30 days after crop emergence. UFFS, Campus Erechim, RS, 2019/2020.

Tabla 4. Parámetros fisiológicos de cultivares de frijol tipo carioca frente al competidor *Urochloa plantaginea*, 30 días después de la emergencia del cultivo. UFFS, Campus Erechim, RS, 2019/2020.

Bean: Competitor	Ci	A	Gs	E	CE	WUE	Dry mass (g pot^{-1})
BRS FC 104							
75:25	265	13.03	0.25	3.12	0.06	4.77	40.92
50:50	287	16.67	0.26	3.46	0.05	4.15	35.45
25:75	258	18.25	0.32	3.59	0.07	5.10	37.15
BRS FC 402							
75:25	272	12.81	0.19	2.60	0.05	4.93	43.55
50:50	284	13.84	0.24	2.92	0.05	4.74	41.22
25:75	264	13.94	0.20	2.67	0.05	5.20	38.88
IAC Imperador							
75:25	287	13.88	0.28	3.63	0.05	3.82	42.29
50:50	263	18.15	0.32	3.93	0.07	4.64	42.51
25:75	274	16.79	0.33	3.96	0.06	4.24	34.39
IAC Milênio							
75:25	303*	11.93	0.24	2.98	0.04	4.01	44.03
50:50	280	9.21*	0.17	2.40	0.04	4.48	36.75
25:75	281	7.26*	0.13	2.01	0.03	4.32	15.91*
IPR Tangará							
75:25	278	15.32	0.34	3.16	0.06	5.19	43.81
50:50	269	19.21	0.37	3.46	0.07	5.62	35.57*
25:75	268	16.98	0.30	3.08	0.06	5.59	32.86*
SCS 205 Riqueza							
75:25	287	13.88*	0.28	3.63	0.05	3.82	40.92
50:50	263	19.35	0.32	3.93	0.07	4.64	35.45
25:75	274	16.79	0.33	3.96	0.06	4.24	37.15

* Significant difference against the competitor, according to the Dunnett's ($p \leq 0.05$). Values into brackets represent the mean standard error. Ci (CO_2 concentration), A (Photosynthetic activity) Gs (stomatal conductance), E (Transpiration rate), CE (carboxylation efficiency) and WUE (efficiency in the use of water).

* Diferencia significativa frente al competidor, según Dunnett's ($p \leq 0,05$). Los valores entre paréntesis representan el error estándar medio. Ci (concentración de CO_2), A (actividad fotosintética) Gs (conductancia estomática), E (tasa de transpiración), CE (eficiencia de carboxilación) y WUE (eficiencia en el uso del agua).

In general terms (table 4), most bean cultivars tended to increase the photosynthesis rate as the proportion of the competitior was also increased. Although this is a sign of a superior competitive ability, it is not the determing factor in choosing carioca bean cultivars with a superior competitive ability against Alexandergrass. Superior water conductance was reported for IAC Imperador, IPR Tangará, and SCS 205 Riqueza. Cultivars IAC Imperador and SCS 205 Riqueza tended to have an overall superior physiological performance in the competition with Alexandergrass. On the down side, IAC Milênio tended to present a lower photosynthetic rate coupled to a superior internal CO_2 as well as a lower carboxilative efficiency (table 4), making it the worst performing cultivar with regard to the physiological aspects.

Conclusions

There is competition between carioca bean cultivars and the weed Alexandergrass for the same environmental resources. From the physiological point of view, the best performing cultivars in the competition with Alexandergrass were IAC Imperador and SCS

205 Riqueza, while the worst was IAC Milênio. The adoption of an ecophysiological strategy of escape to competition when growing carioca beans in Alexandergrass-infested areas is recommended. In other words, the weed should be controlled earlier in the crop cycle, and competitive cultivars such as IAC Imperador and SCS 205 Riqueza should be preferred to reduce losses by competition.

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Physical-chemical properties of spray syrup in tank-mixing multiple pesticides and water sources used in grain farming

Propiedades fisicoquímicas del caldo de pulverización preparado con diferentes plaguicidas y tipos de agua utilizadas durante la producción de granos

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Abstract

This work evaluated physical-chemical properties of spraying syrup formulated with different water types and using or not tank-mixing pesticides. The experimental design was completely randomized in a 4 x 5 factorial scheme (4 water types and 4 pesticide syrups + control, non-sprayed plant) in three sampling times (off-season corn, pre-planting soybean weed control, and soybean season) within one year. Water treatments consisted of deionized, well, river and weir water. Product mixing consisted of herbicide (Glyphosate 925 g a.i. ha^{-1}), herbicide + insecticide (Imidacloprid + beta-cyfluthrin 100+12.5 g a.i. ha^{-1}), herbicide + fungicide (Trifloxystrobin + Prothioconazole 70+60 g a.i. ha^{-1}) and herbicide + insecticide + fungicide. Weir water presented the worst physical quality. Spraying syrup prepared with tank-mixing herbicide, insecticide, and fungicide in various combinations decreased the effectiveness of the pesticide. When associating the three pesticides, less dissolution of the spray syrup and greater risk of syrup incompatibility leads to pesticide ineffectiveness.

Keywords

application technology • water types • plant protection

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Resumen

Este trabajo evaluó las propiedades fisicoquímicas del caldo de pulverización formado con diferentes tipos de agua, con y sin utilizar la mezcla de múltiples plaguicidas. El diseño experimental fue completamente aleatorio, los tratamientos se organizaron en un esquema factorial 4x5 (4 tipos de agua y 4 caldos de plaguicidas + 1 sin producto fitosanitario) en tres épocas de muestreo (maíz de segunda zafra, pre-siembra de la soja y durante cultivo de la soja) en un año. Los tipos de agua fueron agua desionizada, agua de pozo, agua de río y agua de laguna, y la mezcla de productos consistió en herbicida (glifosato 925 g i.a. ha^{-1}), herbicida + insecticida (Imidacloprid + beta-cifluthrina 100 + 12.5 g i.a. ha^{-1}), herbicida + fungicida (Trifloxistrobina + Prothioconazole 70+60 g i.a. ha^{-1}), herbicida + insecticida + fungicida. Se encontró relación entre el origen del agua y la mezcla de los plaguicidas, que influenció la calidad del caldo de pulverización. Cuando el agua proviene de lagunas, el caldo presenta la peor calidad física, lo que disminuye el rendimiento del plaguicida. El caldo con el herbicida, insecticida y fungicida mezclados se diluye menos, aumentando el riesgo de incompatibilidad y de ineficacia de los plaguicidas.

Palabras clave

tecnología de aplicación • tipos de agua • protección vegetal

Introduction

Spraying is widely used in agriculture for plant protection. For pesticide application, a liquid and an active diluted ingredient (phytosanitary product) form a syrup applied towards a target (weed, pest or pathogen) as a homogeneous cloud. The liquid usually used is water, the universal solvent (37). Water quality is essential, as it generally represents around ninety-five per cent of the spray solution. Therefore, chemical and physical elements in water can affect spray quality (18). In this sense, suspended materials such as silt, clay, and organic matter provide a “cloudy” aspect (23).

Soil sorption coefficient (Kd) and soil organic carbon-water partition coefficient (Koc) constitute pesticide-related coefficients that reflect pesticide force to be adsorbed to different particles. Pesticides with high Kd or Koc values bind strongly to sediments and organic matter in water, reducing active ingredients in the solution and reducing effectiveness (12). In this sense, several elements can influence application efficiency and effectiveness, such as Calcium, Magnesium, and Iron, free ions in the spray syrup (38). Accordingly, water hardness is a measure of total Calcium and Magnesium in water, reducing. pesticide effectiveness (13). Considering ion content, the pH also influences active ingredient availability in spray syrup. Acid/base ionization constants (pKa or pKb) represent ionization trends in a given pH range, determining syrup ingredient concentrations in the ionizable form directly affecting product effectiveness (12). Waters from rural regions may present dissolved ions or salts. River and weir waters have sediments such as clay and organic matter that can clog nozzles and filters, reducing spray components life (30). Thus, water choice is important for efficient spraying, especially considering water obtained from open reservoirs, such as rivers and weirs (20).

Another factor involved in application quality is product association in the sprayer tank. Products used in Integrated Pest Management or plant protection programs do not have a wide enough spectrum of action to effectively control all crop pests, making it necessary to mix products (21). Thus, farmers adopting this practice, face certain difficulties like product dissolving, foaming, precipitation, and flocculation, among other physical and chemical incompatibilities (15, 16).

Given these facts, this work evaluated spraying syrup physical-chemical properties when formulated with different water qualities and tank-mixing multiple pesticides.

Material and methods

The experiments were conducted in laboratory and field. Field evaluations were located in the southern State of Mato Grosso do Sul, 22°27'04" latitude S and 55°01'27" longitude W, in Caarapã district, located in the Laguna Carapã city, Brazil. The climate is Monsoon (Am), according to Köppen's classification (3), with dry winters, average annual rainfall of 1500 mm, and average temperature of 22°C (figure 1).

Adapted from National Institute of Meteorology (INMET, https://portal.inmet.gov.br/).

Adaptado del Instituto Nacional de Meteorología (INMET, https://portal.inmet.gov.br/).

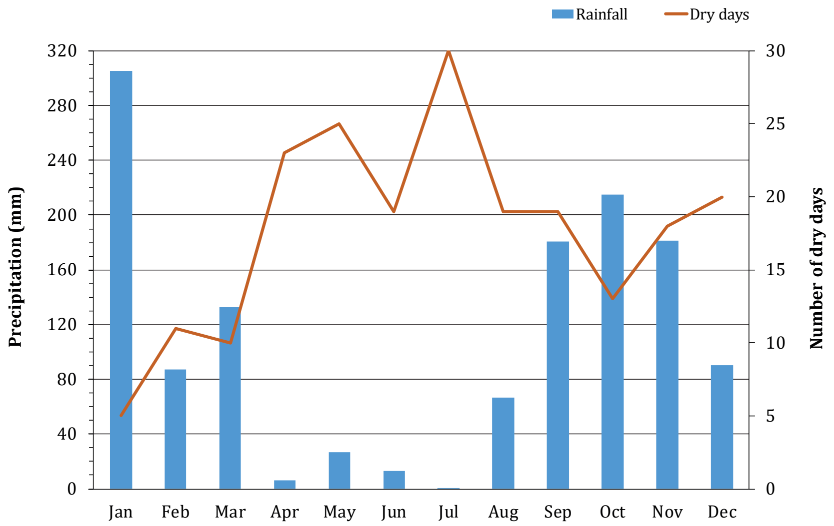

Figure 1. Monthly rainfall and dry days for the Laguna Carapã, Brazil, 2018.

Figura 1. Lluvias mensuales y días secos para Laguna Carapã, Brasil, 2018.

The soil is an Oxisol, with the chemical properties of a Clay soil (0 to 20 cm) under no-tillage (table 1). Soil sampling was carried out at Santa Lúcia farm, located in Laguna Carapã, Brazil.

Table 1. Chemical soil analysis of the Santa Lúcia farm (0 to 20 cm) in Laguna Carapã, Brazil, 2018.

Tabla 1. Análisis químico del suelo de la granja Santa Lúcia (0 to 20 cm) en Laguna Carapã, Brasil, 2018.

pH	OM	P	S	K	Ca	Mg	Al	H+Al	SB	CEC	V	M
$CaCl_2$	g dm^{-3}	mg dm^{-3}		---------------------- Cmol dm^{-3} ----------------------							---- % ----	
5.2	31.1	18.3	4.4	0.3	6.4	1.4	0.0	4.5	8.1	12.6	64.2	0.0

pH: Hydrogen ion concentration; OM: Soil Organic Matter; P: Phosphorus; S: Sulfur; K: Potassium; Ca: Calcium; Mg: Magnesium; Al: Aluminum; H+Al: Potential Acidity; SB: Sum of Bases; CEC: Cation exchange capacity); V: CEC Base Saturation; M: CEC Aluminum Saturation.

pH: Concentración de iones de Hidrógeno; OM: Materia Orgánica del Suelo; P: Fósforo; S: Azufre; K: Potasio; Ca: Calcio; Mg: Magnesio; Al: Aluminio; H+Al: Acidez potencial; SB: Suma de bases; T: Capacidad de intercambio catiónico; V: Saturación de bases; M: Saturación de Aluminio.

The experimental design was completely randomized, in a 4x5 factorial scheme, with four replications. The treatments combined four water qualities (well, river, weir, and deionized water) and five pesticide tank-mixings (3 associations, one with herbicide and an untreated control). All treatments are described in table 2 (page 135).

Table 2. Treatments combining water quality and pesticide tank-mixing.
Tabla 2. Tratamientos con las combinaciones de tipos de agua y productos fitosanitarios.

Treatment	Description
1	Deionized water
2	Deionized water with herbicide
3	Deionized water with tank-mixing1*
4	Deionized water with tank-mixing2*
5	Deionized water with tank-mixing 3*
6	Well water
7	Well water with herbicide
8	Well water with tank-mixing1
9	Well water with tank-mixing2
10	Well water with tank-mixing3
11	River water
12	River water with herbicide
13	River water with tank-mixing1
14	River water with tank-mixing2
15	River water with tank-mixing3
16	Weir water
17	Weir water with herbicide
18	Water weir with tank-mixing1
19	Water weir with tank-mixing2
20	Water weir with tank-mixing3

* Tank-mixing 1: herbicide + insecticide; tank-mixing 2: herbicide + fungicide; tank-mixing 3: herbicide + insecticide + fungicide.

* Mezcla de tanque1: herbicida + insecticida; mezcla 2: herbicida + fungicida; mezcla 3: herbicida + insecticida + fungicida.

Table 3 describes phytosanitary products used in spray syrups, formulation, dosage, and composition of active ingredients. These pesticides were selected for being the most used in the region.

Table3. Characterization of phytosanitary products.
Tabla 3. Caracterización de productos fitosanitarios.

Trade Name	Composition	Formulation	Function	Dosage (g a.i. ha^{-1})
FOX*	Trifloxystrobin + Prothioconazole	SC	Fungicide	70+60
Roundup*	Glyphosate isopropylamine salt; N-phosphonomethyl glycine	SL	Herbicide	925
CONNECT*	Imidacloprid + beta-cyfluthrin	SC	Insecticide	100+12.5

* AUREO: Soy methyl ester (Adjuvant), 0.25%vv; SL: soluble concentrate; SC: flowable suspension concentrate.

* AUREO: Éster de soja metilado (coadyuvante), 0.25%vv; SL: concentrado soluble; SC: suspensión concentrada.

The experiment was conducted in three sampling periods within a year. The first sampling corresponded to the off-season corn crop analyzed in April 2018. The second sampling was done during the pre-planting soybean weed control, while laboratory analyses were performed in September 2018. The third sampling corresponded to the soybean crop season, and the analyses were conducted in November 2018. All samples were analyzed on sampling date.

Three collecting sites were defined for water sampling, considering the spots where farmers usually obtain water from. These sites correspond to river water (22°26'3.45" S; 55°3'54.26" W), well water (22°27'8.79"S; 55°3'6.74" W), and weir water (22°29'8.42" S; 55°3'32.00" W). In addition, deionized water was used as a standard for comparison. Deionized water was produced by Outletlab equipment. The samples were put in 20 L thermoplastic boxes, labelled, immediately transported to the Physical-Chemical Laboratory, and stored in a refrigerated environment.

The following characteristics determined syrup quality: pH, electrical conductivity, turbidity, total dissolved solids, and total solids.

Water samples were separated into Beckers's griffin cup type, with a 500 mL capacity. Half the volume (250 mL) was filled with water. Then, pesticides were added, and the container was completely filled with water, constituting the spraying syrup. The syrups were mixed and homogenized manually using a glass stick simulating the sprayer tank. After mixing, 4 repetitions were subdivided. Control treatments (without pesticide) and water types were put in scaled glass containers (beakers) and homogenized. Mineral oil was added only in fungicide treatments. Pesticides were added according to Abnt (2014).

Physical-chemical characteristics of spray syrups associated with phytosanitary products were determined using standard methods (5), while equipment was tested as described in table 4.

Table 4. Methods and equipment used in laboratory analyses.

Tabla 4. Métodos y equipos utilizados en los análisis de laboratorio.

Characteristic	Equipment	Method
Electrical conductivity	Metrohm 712 Conductometer	Electrometric
pH	Metrohm pH meter	Potentiometric
Total dissolved solids	Metrohm 712 Conductometer	Specific conductance
Turbidity	Instrutherm TD 200 Turbidimeters	Nephelometric
Total Solids	Drying chamber	Gravimetric

The physical-chemical data of spraying syrup were submitted to ANOVA, and the means were compared using the Tukey test, at 5% probability, Sisvar software (14).

Results and discussion

Significant interactions ($p<0.05$) were found between water qualities and product associations, regardless of sampling time. Total solids showed a significant influence of the tank-mixing multiple pesticides. Treatments with three classes of phytosanitary products (herbicide + insecticide + fungicide) presented the highest content of total solids, regardless of water quality throughout sampling times. The herbicide + fungicide and herbicide + insecticide + fungicide tank-mixing treatment also showed the highest concentration of solids in the off-season corn crop and with well water (table 5, page 137).

Table 5. Water quality and pesticides association for total solids (mg L^{-1}).
Tabla 5. Sólidos totales (mg L^{-1}) considerando las interacciones*entre los tipos de agua y la mezcla de plaguicidas.

Spraying syrup	Water type			
	Deionized	Well	River	Weir
Off-season corn crop(1)				
Herbicide + insecticide + fungicide	25981 aA	24435 bA	25914 aA	26360 aA
Herbicide + fungicide	22080 cB	24776 baA	23802 bB	25310 aB
Herbicide + insecticide	21109 cB	22364 baB	21432 cbC	22807 aC
Herbicide	17544 cC	18408 bC	18515 bD	19928 aD
Non-defensive	2 bD	11 bD	58 bE	1380 aE
Pre-planting soybean weed control(2)				
Herbicide + insecticide + fungicide	26300 bA	25638 bA	26322 bA	27401 aA
Herbicide + fungicide	21434 aB	19962 cC	20741 bC	21001 baC
Herbicide + insecticide	21215 bB	22070 aB	22198 aB	22366 aB
Herbicide	17544 cC	18408 bD	18515 bD	19928 aD
Non-defensive	2 aD	14 aE	64 aE	574 aE
Season soybean crop(3)				
Herbicide + insecticide + fungicide	24012 bA	28652 aA	29124 aA	28604 aA
Herbicide + fungicide	21521 bB	23114 aB	23595 aC	21668 bB
Herbicide + insecticide	19960 dC	22971 bB	25798 aB	21715 cB
Herbicide	17036 cD	19969 bC	20658 baD	20852 aB
Non-defensive	2 bE	83 bD	112 bE	1483 aC

*F-test ($p<0.05$). Coefficient of variation = 2.88(1), 2.10(2) and 2.36(3)%. Different lowercase letters in the row and uppercase letters in the column indicate statistical for Tukey test, $p \leq 0.05$.

*Prueba F ($p<0,05$). Coeficiente de variación = 2,88(1), 2,10(2)y 2,36(3)%. Diferentes letras minúsculas en la fila y mayúscula en la columna indican estadística para la prueba de Tukey, $p \leq 0,05$.

In this sense, product association in the sprayer tank increased total solids, regardless of sampling times and water quality (table 5). When phytosanitary products are mixed in the spray tank, physicochemical interactions given by unknown incompatibilities (two or more mixed chemicals suffer physical-chemical changes) may occur (37). This incompatibility can result in antagonistic effects and consequent reduced effectiveness of pesticides.

In control treatments, the highest total solids were found for weir water in the first and third seasons. However, no interaction was found between water qualities in the second sampling, probably given to absent rain (figure 1, page 134). Similarly, water uptake influenced total solids in the spray syrup, also aggravated by low precipitation during the period. In the second period, all treatments with deionized water presented the lowest values for this variable, statistically differing from the other sources. Regardless of the season, a tendency for increased solids was observed in weir water treatments.

In the driest period, dissolved solids from drag dam and river edges resulted in greater concentration (2) generating greater turbidity. In the wettest period, these solids tended to be in lower concentrations. According to Farias *et al.* (2014), open-air sources, such as rivers and dams, are affected by rainwater and winds, carrying sediments such as clays. However, unlike weirs, rivers do not present still water, and thus, higher loading and dissolution of sediments reduced this water´s total solids values (37).

Solids in the syrup can inactivate glyphosate (20), which is adsorbed onto clay, reducing plant absorption and efficacy (24). Ramos and Durigan (1998) evaluated weir water influence on post-emergent herbicide efficacy. They found that solutions formulated with up to 10 g L^{-1} of soil from the Jaboticabal region containing 56% clay did not interfere with glyphosate efficacy. Brazilian soils are composed of low-activity clay, such as kaolinite.

This means that cloudy water composition also impacts pesticide effectiveness. In tropical regions, such as the one in the present study, a higher concentration of organic matter (OM) in water, with greater effective cation exchange capacity, may result in greater adsorption of pesticides by OM than by clay minerals.

Analysis of total dissolved solids (table 6) showed a precipitating tendency of products with the herbicide treatment, while the treatments with the association of three phytosanitary products (herbicide + insecticide + fungicide) showed lower dissolved solids between mixtures, regardless of water quality and sampling times analyzed, excepting well water at the off-season corn sampling. These results indicate that when different phytosanitary products are associated, a higher content of solids in the sprayer tank may cause clogging of nozzles and filters.

Table 6. Water types and products association for total dissolved solids (mg L^{-1}).
Tabla 6. Sólidos disueltos totales (mg L^{-1}) considerando las interacciones* entre los tipos de agua y la mezcla de plaguicidas.

Spraying syrup	Water type			
	Deionized	Well	River	Weir
Off-season corn(1)				
Herbicide + insecticide + fungicide	6613 cC	6791 bB	6740 bD	7073 aA
Herbicide + fungicide	6874 cbB	6986 aA	6829 cC	6918 bB
Herbicide + insecticide	6644 bC	6958 aA	6986 aB	6969 aB
Herbicide	6955 bA	6981 bA	7090 aA	7085 aA
Non-defensive	2,8 aD	27,0 aC	24,2 aE	12,2 aC
Pre-planting soybean weed control(2)				
Herbicide + insecticide + fungicide	6542 cB	8712 bC	9151 aB	9174 aB
Herbicide + fungicide	6603 cBA	9083 bB	9038 bB	9362 aB
Herbicide + insecticide	6628 cBA	9144 bBA	9078 bB	9266 aB
Herbicide	6758 cA	9283 bA	9347 bA	9687 aA
Non-defensive	3 aC	21 aD	38 aC	26 aC
Season soybean crop(3)				
Herbicide + insecticide + fungicide	6618 bC	6623 bD	6621 bB	6900 aC
Herbicide + fungicide	6702 bCB	7007 aB	6658 bBA	6971 aCB
Herbicide + insecticide	6733 bB	6735 bC	6601 cB	7030 aB
Herbicide	6908 bA	7227 aA	6763 cA	7217 aA
Non-defensive	4 aD	29 aE	49 aC	33 aD

*F-test ($p<0.05$). Coefficient of variation = 0.56(1), 1.44(2) and 1.02(3)%. Different lowercase letters in the row and uppercase letters in the column indicate statistical for Tukey test, $p \leq 0.05$.

*Prueba F ($p<0,05$). Coeficiente de variación = 0,56(1), 1,44(2) y 1.02(3)%. Diferentes letras minúsculas en la fila y mayúscula en la columna indican estadística para la prueba de Tukey, $p \leq 0,05$.

Petter *et al.* (2012) and Pazini *et al.* (2013), evaluating association compatibility of different classes of phytosanitary products, concluded that depending on the added products, the association of more than one type of pesticide can cause physical incompatibility and low dissolution in the spray tank. According to these authors, this incompatibility is due to the type of formulation of phytosanitary products.

The concentrated suspension (SC) formulation is not always stable, and in resting syrup, solid particles can settle (19, 37). When associating phytosanitary products with the same kind of formulation, there is hardly any incompatibility in the syrup. However, this rarely happens in the field. The treatment without any association (herbicide only) showed higher total dissolved solids, due to the high solubility of glyphosate in water (15,700 mg L^{-1} at 25°C and pH 7 - acid) (31), justifying the higher dissolved solid concentration in syrup.

Mean total dissolved solids obtained in the non-defensive treatments were similar between water qualities in the three sampling times (table 6, page 138). Besides, dissolved solids alter water appearance, as turbidity (29). Both insecticide and fungicide increased spray syrup turbidity (table 7).

Table 7. Water types and association of products for syrup turbidity.
Tabla 7. Turbidez del caldo de pulverización considerando las interacciones* entre los tipos de agua y la mezcla de plaguicidas.

Spraying syrup	Water type			
	Deionized	Well	River	Weir
Off-season corn crop(1)				
Herbicide + insecticide + fungicide	5163 dA	8177 cA	8540 bA	9401 aA
Herbicide + fungicide	4597 dB	6847 cB	8052 bB	9073 aB
Herbicide + insecticide	1811 bC	1369 dC	1767 cC	2460 aC
Herbicide	1 bD	6 bD	26 bD	281 aD
Non-defensive	1 bD	6 bD	18 bD	211 aE
Pre-planting soybean weed control(2)				
Herbicide + insecticide + fungicide	5728 aA	3229 bA	3284 bA	5714 aA
Herbicide + fungicide	3490 aB	1885 cB	2444 bB	3606 aB
Herbicide + insecticide	2425 aC	1542 cC	1711 bC	2517 aC
Herbicide	8 bD	2 bD	25 bD	575 aD
Non-defensive	3 bD	1 bD	17 bD	354 aE
Season soybean crop(3)				
Herbicide + insecticide + fungicide	8642 dA	9411 cA	10581 bA	10896 aA
Herbicide + fungicide	4338 dB	4942 cB	8614 bB	9098 aB
Herbicide + insecticide	3389 aC	3460 aC	3509 aC	3539 aC
Herbicide	11 bD	24 bD	37 bD	654 aD
Non-defensive	4 bD	13 bD	28 bD	549 aD

*F-test ($p<0.05$). Coefficient of variation = 0.45(1), 3.81(2) and 2.85(3)%. Different lowercase letters in the row and uppercase letters in the column indicate statistical for Tukey test, $p \leq 0.05$.

*Prueba F ($p<0,05$). Coeficiente de variación = 0,45(1), 3,81(2) y 2,85(3)%. Diferentes letras minúsculas en la fila y mayúscula en la columna indican estadística para la prueba de Tukey, $p \leq 0,05$.

Spray syrups made with weir water, including those treatments without pesticides, were the ones with the highest turbidity values, regardless of product association. As already mentioned, water quality for agricultural spraying is closely related to spray physical quality considered as suspended sediment content. When analyzing waters without pesticides in all sampling periods, higher concentrations of dissolved solids were observed in weir water, representing a greater potential for interaction with pesticides, and consequent less effectiveness.

Treatments with only herbicide did not differ from the treatments without pesticides (water only) regarding turbidity, except for the herbicide treatment with weir water in the first and second sampling times. Considering turbidity may be influenced by product formulation, soluble formulations such as glyphosate, highly soluble in water (4), generate lower turbidity. In contrast, concentrated suspension formulation (fungicide and insecticide) with a milky appearance, increase syrup turbidity (photo, page 140).

When observing mixtures of the herbicide lactofen (Drible® 240 - formulation CE - *emulsifiable concentrate*) with the insecticides Methomy Chlorpyrifos, Cypermethrin, Thiamethoxam/Lambdacialotrine, Teflubenzuron, and Triflumurom, Petter *et al.* (2012) found physical incompatibility in syrups prepared with water, water with pylyrolean acid and water with boric acid. This incompatibility varied between grade 2 (separation after 1 minute, not to be applied) and grade 4 (separation after 10 minutes, apply on continuous agitation), resulting in non-homogeneous mixing, with a decanting tendency. Before, Petter *et al.* (2007) had observed physical-chemical interaction caused by CE with Chlorpyrifos SC (flowable suspension concentrate). According to Theisen and Ruedell (2004), most physical and chemical incompatibilities are observed in mixtures of products with CE formulations and WP (Wettable Powders), EW (emulsion in water) and SC.

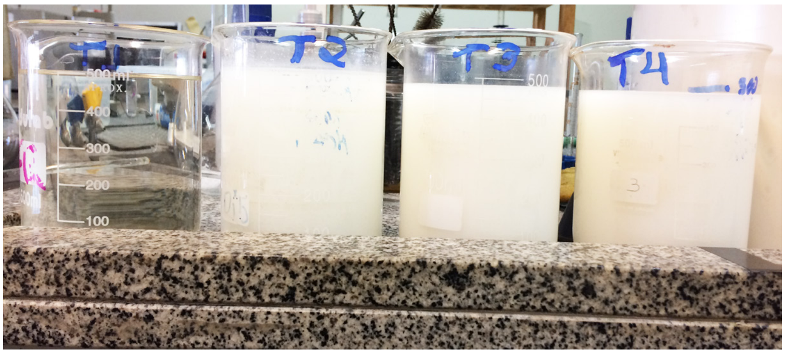

Photo. Turbidimetry of the phytosanitary products in deionized water. T1: Herbicide; T2: Herbicide + Insecticide; T3: Herbicide + Fungicide; T4: Herbicide + Insecticide + Fungicide.

Foto. Turbidimetría de los productos fitosanitarios en agua desionizada. T1: Herbicida; T2: Herbicida + Insecticida; T3: Herbicida + Fungicida; T4: Herbicida + Insecticida + Fungicida.

In this study, product association altered spraying syrup pH (table 8). This variation was lower in the soybean pre-planting weed control sampling. Treatments with phytosanitary products did not differ from each other, except for well water. However, no abrupt change between any treatments occurred, nor did they present alkaline pH (pH> 7), which according to Vuković *et al.* (2013), causes syrup instability for acidic products.

Table 8. Water types and products association for pH of spraying syrup.
Tabla 8. pH del caldo de pulverización considerando las interacciones* entre los tipos de agua y la mezcla de plaguicidas.

Spraying syrup	Water type			
	Deionized	Well	River	Weir
Off-season corn crop[1]				
Herbicide + insecticide + fungicide	6.17 cA	6.40 bBA	6.37 bC	6.67 aA
Herbicide + fungicide	6.05 cBA	6.32 bBA	6.55 aB	6.47 aB
Herbicide + insecticide	5.98 cB	6.27 bB	6.52 aB	6.47 aB
Herbicide	6.10 cBA	6.42 bA	6.57 aB	6.45 bB
Non-defensive	6.02 cB	5.65 dC	7.00 aA	6.47 bB
Pre-planting soybean weed control[2]				
Herbicide + insecticide + fungicide	6.05 aA	4.67 bB	4.85 bA	4.75 bA
Herbicide + fungicide	6.05 aA	4.80 bBA	4.82 bA	4.72 bA
Herbicide + insecticide	5.97 aA	4.95 bBA	4.87 bA	4.82 bA
Herbicide	6.22 aA	5.05 bA	4.80 bA	4.77 bA
Non-defensive	6.12 aA	4.62 bB	4.77 bA	4.65 bA
Season soybean crop[3]				
Herbicide + insecticide + fungicide	6.27 aA	5.65 bA	5.60 bB	5.67 bA
Herbicide + fungicide	6.07 aA	5.65 bA	5.55 bB	5.60 bA
Herbicide + insecticide	6.07 aA	5.55 bBA	5.75 bBA	5.57 bA
Herbicide	6.17 aA	5.80 bA	5.67 bBA	5.75 bA
Non-defensive (control)	6.05 aA	5.25 cB	5.95 baA	5.72 bA

*F-test ($p<0.05$). Coefficient of variation = 1.03[1], 3.34[2] and 2.82[3]%. Different lowercase letters in the row and uppercase letters in the column indicate statistical for Tukey test, $p \leq 0.05$.

*Prueba F ($p<0{,}05$). Coeficiente devariación=1,03[1],3,34[2] y 2,82[3] %. Diferentes letras minúsculas en la fila y mayúscula en la columna indican estadística para la prueba de Tukey, $p \leq 0{,}05$.

During pre-plating soybean weed control and soybean crop, no statistical difference between treatments was observed for deionized and well water, *i.e.*, product addition did not influence spray pH (table 8, page 140). Deionized water is pure water that has undergone a process of filtration and total removal of ions like nitrate, Calcium, and Magnesium, among other elements (32). This is considered ideal for spray syrup water, precisely for keeping water quality indicators, such as pH (19). On the other hand, the non-influence of weir water on pH is related to a higher concentration of suspended particles than ions, thus affecting attributes like dissolved solids and solids before affecting pH. For well water, during the three sampling times, an increase in pH was observed after the addition of phytosanitary products, except for the association between herbicide + insecticide + fungicide, in the soybean pre-planting weed control sampling, the driest period. Another relevant fact is that, regardless of sampling time, when glyphosate was added to well water, the highest pH increase was observed. Typically, well waters have high ion concentrations, especially: Ca^{2+}, Mg^{2+}, Na^{+}, $K^{+,}$ HCO_3^{-}, $CaSO_4^{2-}$.

Well waters are often classified as hard waters, with high $CaCO_3$ concentrations. Variations in average hardness are related to soil geological nature. Due to high $CaCO_3$ concentration, well water usually reports alkaline pH (7, 36, 39). The use of this hard water may cause ion chelation by glyphosate (34), decreasing efficiency. In addition, given well water alkalinity and glyphosate acid nature, this herbicide is dissociated, losing efficiency (30). Additionally, after mixing phytosanitary products, the resulting syrup may present different pH values. In general, association incompatibility occurs with high syrup pH alteration, leading to degradation of active ingredients and consequent undesirable chemical compounds (37).

Water pH IS The main parameter influencing spray syrup compatibility (11). Ideal pH for plant protection varies among products (37). Pyrethroids insecticides have a pH efficiency of around 4. Carvalho *et al.* (2009) report syrup pH ranging from 4.6 to 5 for glyphosate, while Schilder (2008) mentions 5 to 6. Cunha *et al.* (2017) observed that the combination of Trifloxystrobin + Prothioconazole had pH 7, while with the adjuvant soy methyl ester, achieved pH 5. Most phytosanitary products, in the presence of alkaline pH (>7), decompose rapidly causing in-tank associations instability (17).

Syrup mean electrical conductivities (EC) differed among all treatments (table 9, page 142). Glyphosate highly influenced spray syrup EC. The more acidic the water, the greater the electrical conductivity measured in the syrup after glyphosate addition. According to Amarante Júnior *et al.* (2002), glyphosate is a strong acid with a pH between 2.2 and 5.4, with two dissociations, and a pH 5.5 with three dissociations and an increasing EC.

Evaluating surface tension, pH, and EC of phytosanitary syrup, Cunha *et al.* (2017) demonstrated that glyphosate presented the highest conductivity among tested products using deionized water, evidencing that water addition influences spray syrup EC. In this study, glyphosate EC increased at pH above 6.3. Similarly, with the tank-mixing multiple pesticides, a decrease in syrup EC was observed when compared with the treatment without association, proving that tank-mixing causes chemical interaction between the products (39). Thus, decreasing EC was measured in the three sampling times and with distinct pH ranges between seasons.

Regarding non-defensive treatments, no difference was found between water qualities at any time of analysis. These results differed from those found by Rheinheimer and Souza (2000) and Farias *et al.* (2014) when verifying low EC values in weir water and higher in well water.

Electrical conductivity represents the solution ion concentration that can conduct electricity. Higher conductivity values express higher number of ions in the solution. Thus, the greater the possibility of these ions interacting with agrochemical molecules, the greater the incompatibility of the syrup (8). However, there is no default value for EC, as this characteristic depends on the application volume. According to Vargas *et al.* (1997), smaller application volumes cause less EC influence on herbicides. *i.e.*, decreasing ion proportion concerning pesticide molecules. Considering the same concentration, using an application rate of 1.0 L ha^{-1}, allows lower ion interference (EC) than an application using 30 L ha^{-1} (6).

In this context, this study showed a tendency to increase product dissolution in the spray syrup for no products association and when using high quality water, such as deionized water.

Table 9. Water types and products association for electrical conductivity (µS cm^{-1}).
Tabla 9. Conductividad eléctrica (µS cm^{-1}) considerando las interacciones* entre los tipos de agua y la mezcla de plaguicidas.

Spraying syrup	Water type			
	Deionized	Well	River	Weir
Off-season corn crop(1)				
Herbicide + insecticide + fungicide	10020 cB	13200 bC	13865 aB	13900 aB
Herbicide + fungicide	10415 cbA	13762 bB	13695 bB	14185 aB
Herbicide + insecticide	10067 bB	13855 baBA	13755 bB	14040 aB
Herbicide	10537 cA	14065 bA	14162 bA	14677 aA
Non-defensive	4 aC	32 aD	58 aC	40 aC
Pre-planting soybean weed control(2)				
Herbicide + insecticide + fungicide	9912 cC	10290 bB	10212 bD	10717 aA
Herbicide + fungicide	10005 cCB	10585 aA	10347 bC	10482 aB
Herbicide + insecticide	10042 cB	10542 aA	10585 aB	10560 aB
Herbicide	10240 cA	10577 bA	10742 aA	10735 aA
Non-defensive	4 aD	41 aC	36 aE	18 aC
Season soybean crop(3)				
Herbicide + insecticide + fungicide	10027 bC	10035 bD	10032 bB	10455 aC
Herbicide + fungicide	10155 bCB	10617 aB	10087 bBA	10562 aCB
Herbicide + insecticide	10202 bB	10205 bC	10002 cB	10652 aB
Herbicide	10467 bA	10950 aA	10247 cA	10935 aA
Non-defensive	5 aD	43 aE	74 aC	50 aD

* F-test ($p<0.05$). Coefficient of variation = 1.38(1), 0.76(2) and 1,02(3)%. Different lowercase letters in the row and uppercase letters in the column indicate statistical for Tukey test, $p \le 0.05$.

*Prueba F ($p<0,05$). Coeficiente de variación = 1,38(1), 0,76(2) y 1,02(3)%. Diferentes letras minúsculas en la fila y mayúscula en la columna indican estadística para la prueba de Tukey, $p \le 0,05$.

Phytosanitary product solubility is measured as solubility in water, variable among active ingredients. Glyphosate had the highest water solubility among tested active ingredients by Soares *et al.* (2017). For this reason, correct syrup preparation and association of phytosanitary products in the spray tank, are important (19). However, even following preparation order, treatments associated with tank-mixing multiple pesticides presented worse physical-chemical quality.

Conclusions

Water from different sources and tank-mixing multiple pesticides affect spraying syrup quality. Weir water presents the worst physical quality among the tested sources. It may decrease pesticide performance.

Spraying syrup prepared with tank-mixing herbicide, insecticide, and fungicide in various combinations increased in the successive cultivation of soybean/corn in the studied area. When the three pesticides are associated, less dissolution of the spray syrup and a greater risk of syrup incompatibility takes place, rising fungicide ineffectiveness.

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Nutraceutical effects of organic Selenium and vitamin E supplementation on performance, antioxidant protection and egg quality of Japanese quails (*Coturnix japonica*)

Efectos nutracéuticos de la suplementación con Selenio orgánico y vitamina E sobre el rendimiento, la protección antioxidante y la calidad del huevo de codornices japonesas (*Coturnix japonica*)

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Abstract

This study evaluated the nutraceutical effects of organic Selenium and vitamin E supplementation on performance, egg quality and antioxidant protection of Japanese quails. Forty-two posture cages with six birds each were randomly set in seven treatments. Each treatment consisted of the addition of 200 IU of vitamin E/kg of feed and increasing levels of organic Selenium. Significant differences were found in α-Tocopherol deposition, enzymatic activity of glutathione peroxidase (GSH-Px) and oxidative bioindicator malondialdehyde (MDA) in egg yolk with vitamin E supplementation. We concluded that supplementing 200mg of vitamin E and 0.30 ppm organic Selenium did not affect bird performance, but improved egg quality and shelf life.

Keywords
α-Tocopherol • glutathione peroxidase • malondialdehyde

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Resumen

Este estudio evaluó los efectos nutracéuticos de la suplementación con Selenio orgánico y vitamina E sobre el rendimiento, la calidad del huevo y la protección antioxidante de las codornices japonesas. Cuarenta y dos jaulas de postura con seis aves cada una se colocaron al azar en siete tratamientos. Cada tratamiento consistió en la adición de 200 UI de vitamina E/kg de alimento y niveles crecientes de Selenio orgánico. Se encontraron diferencias significativas en la deposición de α-tocoferol, la actividad enzimática de la glutation peroxidasa (GSH-Px) y el bioindicador oxidativo malondialdehído (MDA) en yema de huevo con suplementos de vitamina E. Concluimos que la suplementación con 200 mg de vitamina E y 0,30 ppm de Selenio orgánico no afectó el rendimiento de las aves, pero mejoró la calidad del huevo y la vida útil.

Palabras clave

α-tocoferol • glutation peroxidasa • malondialdehído

Introduction

Tocopherols and Selenium are important antioxidants that inhibit lipid peroxidation and free radical formation, fighting cell damage and preventing cancer and heart diseases (25). Selenium, a crucial trace element, is an important constituent of several selenoproteins. It regulates the synthesis of certain key antioxidant enzymes called glutathiones. Among these enzymes, the widely studied glutathione peroxidase (GSH-px), detoxifies peroxides and hyperperoxides. Further, Sodium selenite and selenomethionine (an organic form that can be biologically metabolized by animals), constitute a principal sources of Selenium (23).

Vitamin E comprises several compounds called tocopherols. Alpha-tocopherols have important activity in organic antioxidant protection, mainly preventing peroxidation processes of long cell membrane-chain fatty acids (19). In Japanese quails, Akil and Piliang (2012) observed that increasing deposition of tocopherol and Selenium in egg yolk was directly proportional to dietary supplementation levels of these compounds. Additionally, they also discovered a reduction in yolk concentration of malondialdehyde during supplementation of Selenium in combination with vitamin E, thereby causing an increase in hatchability. Conceptually, foods enriched with bioactive compounds are called functional foods or nutraceuticals (11). Due to the innumerable and well-known advantages of quail production, biofortified eggs can be an important functional food. Thus, this study aimed to evaluate the nutraceutical effects of supplementing organic Selenium and vitamin E on performance, egg quality and antioxidant protection of Japanese quails.

Material and Methods

The trial was carried out at the Experimental Poultry House, located in the State of Rio de Janeiro- Brazil, latitude 22°51’08” S, longitude: 43°46’31” W and 13 m a. s. l. A total of 252 Japanese Fujikura female quails with an average weight of 188.32 ± 4 grams and a mean stance of 90%, were utilized. The experiment was approved by the Institution’s Ethics and Biosafety Committee (number 0059/2013).

Lot standardization was subsequently followed by quail random distribution in 42 posture cages (dimensions of 33 x 25 x 20 cm). The experimental design was entirely randomized with seven treatments, six replicates and six birds per cage. The treatments consisted of the addition of 200 mg of vitamin E/kg of feed (DL-α-tocopheryl acetate 99%) and increasing levels of organic Selenium. The experimental rations were obtained from a control diet, with increasing levels of organic Selenium (0.10, 0.20, 0.30, and 0.40 mg of selenomethionine per kilogram of feed) and 200 mg of acetate of DL-alphatocopheryl per kg of diet (as a source of vitamin E). Treatments were as follows: (1) Control diet (CD); (2) CD + 200 mg of vitamin E (VE); (3) CD + 0.20 ppm Selenium yeast (SE); (4) CD + 0.10 ppm Selenium yeast + 200 mg vitamin E (SVE1); (5) CD + 0.20 ppm Selenium yeast + 200 mg vitamin E (SVE2); (6) CD + 0.30 ppm of Selenium yeast + 200 mg of vitamin E (SVE3); (7) CD + 0.40 ppm Selenium yeast + 200 mg vitamin E (SVE4).

According to the nutritional requirements of Japanese quails (16), diets were calculated by Super Crac 5.0 TD package, except for crude protein and calcium, which were based on the recommendations of Oliveira *et al.* (1999) and Barreto *et al.* (2007), respectively. Then, the values of apparent metabolizable energy of corn, soybean meal, and soybean oil were adjusted according to Moura *et al.* (2010). Table 1 shows experimental dietary composition and vitamin and mineral supplementation. Performance variables were mean egg production (%/quail/day), feed consumption (g/quail/day), egg weight (g), egg mass (quail/day), and feed.

Table 1. Nutritional composition of experimental diets.

Tabla 1. Composición nutricional de dietas experimentales.

Nutritional Composition	Experimental diets[1]						
	CD	VE	SE	SVE1	SVE2	SVE3	SVE4
ME ($kcal.kg^{-1}$)[2,3]	2.900	2.900	2.900	2.900	2.900	2.900	2.900
Crude protein ($g.kg^{-1}$)[4]	190	190	190	190	190	190	190
Calcium ($g.kg^{-1}$)[4]	30	30	30	30	30	30	30
Phosphorus ($g.kg^{-1}$)[4]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Lysine ($g.kg^{-1}$)[5]	11	11	11	11	11	11	11
Met + Cys ($g.kg^{-1}$)[5]	7	7	7	7	7	7	7
Sodium ($g.kg^{-1}$)[5]	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Selenium ($mg.kg^{-1}$)[4]	0.07	0,09	0,28	0,16	0,29	0,34	0,45
Vitamin E ($mg.kg^{-1}$)[4]	9,78	217,4	11,58	221,3	227,2	219,7	222,8

[1] Control diet (CD); CD + 200 mg of vitamin E (VE); CD + 0.20 ppm of organic Selenium (SE); CD + 0.10 ppm of organic Selenium + 200 mg of vitamin E (SVE1); CD + 0.20 ppm of organic Selenium + 200 mg of vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm of organic Selenium + 200 mg of vitamin E (SVE4). / [1] Dieta control (CD); CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4).

[2] Metabolizable energy. / [2] Energía metabolizable.

[3] Calculated values. / [3] Valores calculados.

[4] Analized in Animal Nutrition Laboratory - LZO/CCTA/UENF. / [4] Analizado en Laboratorio de Nutrición Animal - LZO / CCTA / UENF.

[5] Analized in Bioagri Alimentos Ltda laboratory. / [5] Analizado en Laboratorio Bioagri Alimentos Ltda.

For egg quality assessment (a) yolk weight (g); (b) albumen weight (g); (c) shell weight (g); (d) percentage of yolk; (e) percentage of shell; and (f) percentage of albumen, were evaluated. Tocopherol and Selenium nutritional effects were evaluated 42 days after supplementation, analytically determining nutrient concentrations. Metabolic indicatorsmalondialdehyde (MDA) in quail egg and blood were determined, according to Shahryar *et al.* (2010) and Enkvetchakul *et al.* (1995): (a) concentration of total yolk tocopherols (μg/g egg); (b) egg bark Selenium, (μg/g), and blood (μg/l); (c) blood MDA (mmol/l) and egg yolk MDA (mmol/g); (d) blood GSH-px (EC 1.11.1) (unit/l).

For further evaluation of Selenium/vitamin E supplementation effects on egg antioxidant protection and consequent shelf life, 36 samples per replicate were stored up to 24 days at 22°C. For this purpose, firstly, all quails were fed with a reference adaptive ration for seven days. Subsequently, they were offered the respective ad libitum experimental rations. Yolk MDA was analyzed every 8 days (1, 8, 16 and 24 days). In addition, the birds were submitted to a photoperiod of 17 hours. Daily temperature and relative humidity were recorded. Feed intake, egg production, weight and mass were measured weekly.

Yolk, albumen, and bark weight were determined by collecting three eggs from each replicate, daily. As a reference, on the experimental "zero" day, 80 eggs were randomly collected and evaluated under the same protocol. In the last four days of the trial, 12 eggs of each replicate were collected and sent to the laboratory for Selenium and vitamin E determinations. For micronutrients analysis, high-performance liquid chromatography (HPLC) was employed, according to Marques *et al.* (2011).

At the end of the trial, three quail blood samples per repetition were collected via the brachial vein. Subsequently, free Selenium, MDA, and glutathione peroxidase (GSH-px) plasma concentrations were determined. Statistical analysis included ANOVA, composite symmetry with a constant correlation between repeated measures over time; autoregressive correlations between repeated measures over time; and variance/covariance unrestricted structure. Model fit was performed using PROC MIXED (SAS System, Inc., Cary, NC, USA).

Performance variables were analyzed by mixed models PROC MIXED, (SAS System, Inc., Cary, NC, USA), and Tukey test. Egg quality, vitamin E and Selenium were analyzed by generalized linear models, using GLIMMIX (SAS System, Inc., Cary, NC, USA), and Tukey test. The mathematical model adopted for mathematical procedures was:

$$Yij = \mu + \alpha j + eij,$$

in which = observation of *Yij* is the j-th treatment in the i-th observation, μ = overall mean, αj= effect of experimental diet level j, and *eij* = random error for each observation *Yij*.

Results

For performance variables, only egg production, feed intake, and egg mass showed significant differences ($p < 0.05$) between treatments. The supplementation of 200 mg of Vit E + 0.40 ppm of organic Selenium in the diet reduced feed intake, probably causing the evidenced loss in egg production and mass (table 2). No significant difference (p > 0.05) was observed in egg quality (table 3).

Table 2. Mean performance values for Japanese quail.

Tabla 2. Valores medios de rendimiento de codorniz japonesa.

Experimental Diet	EP	MFI	EG	AEW	FC
Control diet (CD)	91.59a	29.13a	7.56a	12.13	3.87
VE diet	93.74a	28.33ab	7.52a	12.49	3.78
SE diet	95.58a	27.96ab	7.61a	12.57	3.69
SVE1 diet	90.13a	26.05bc	7.32a	12.33	3.57
SVE2 diet	93.03a	26.30bc	7.34a	12.72	3.61
SVE3 diet	89.46a	24.20cd	7.36a	12.18	3.31
SVE4 diet	77.84b	23.68d	6.18b	12.61	3.86
p - value	0.0397	0.0001	0.0006	0.4824	0.0856

EP-Eggs Production/ MFI- Mean Feed Intake / EG-Egg mass/ AEW- Average Egg Weight/FC- Food Conversion GSH-px: reduced glutathione peroxidase. Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4). Different lower/upper case letters indicate significant differences at p≤ 0.05 for Tukey test.

EP- Producción de huevos / MFI- Consumo medio de alimento / EG-Masa de huevo / AEW- Peso promedio del huevo / FC- Conversión de alimentos GSH-px: Glutation peroxidasa reducida. Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4). Las medias de tratamiento seguidas de diferentes letras en la columna difieren en el nivel de probabilidad de 0.05 según la prueba de Tukey.

Table 3. Mean parameters for egg quality of Japanese quail.

Tabla 3. Media de los parámetros de calidad del huevo de codorniz japonesa.

Experimental Diet	Weight (g)			Percentage (%)		
	Yolk	Shell	Albumen	Yolk	Shell	Albumen
Control diet	3.86	1.03	7.24	31.83	8.49	59.68
VE diet	4.15	1.09	7.25	33.23	8.73	58.04
SE diet	4.00	1.08	7.49	31.81	8.65	59.54
SVE1 diet	3.96	1.08	7.29	32.18	8.75	59.08
SVE2 diet	4.10	1.13	7.49	32.22	8.88	58.91
SVE3 diet	3.91	1.08	7.24	31.98	8.82	59.20
SVE4 diet	3.99	1.10	7.41	32.01	8.86	59.13

Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4).

Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4).

Significant difference (P <0.05) was found for vitamin E supplementation on α-Tocopherol deposition, as well as in GSH-px activity and MDA oxidative bioindicator, as described in table 4, figure 1 and figure 2 (page 150). Adding vitamin E and Selenium, extended shelf life of quail eggs (table 5, page 150).

Table 4. Alpha-tocopherols, Selenium, and malondialdehyde concentration, and glutathione activity in egg and blood of Japanese quails.

Tabla 4. Concentración de alfa-tocoferoles, Selenio, malondialdehído y actividad glutation en huevo y sangre de codornices japonesas.

Diets	αTocopherol	Selenium content			Malondialdehyde	Glutathione
	Yolk (µg/g)	Egg (µg/g)	Shell (µg/g)	Blood (µg/l)	Blood (mmol/l)	
Control diet	38.2d	0.30	0.10	2.76	1.18a	0.64a
VE	588.2a	0.48	0.10	2.84	0.65c	0.68b
SE	37.3d	0.50	0.15	3.83	0.81b	0.80d
SVE1	591.9a	0.48	0.15	3.38	0.51d	0.70c
SVE2	564.1ab	0.62	0.15	3.78	0.43e	0.96e
SVE3	512.5bc	0.75	0.25	4.08	0.38f	1.13f
SVE4	486.1c	0.73	0.33	4.67	0.33g	1.19g
p - value	0.00014	0.8427	0.9551	0.0875	0.0008	0.00259

GSH-px: reduced glutathione peroxidase. Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4). Different lower/upper case letters indicate significant differences at p≤ 0.05 for Tukey test.

GSH-px: glutation peroxidasa reducida. Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4). Las medias seguidas de diferentes letras em columnas o líneas, difieren en el nivel de probabilidad de 0,05 según la prueba de Tukey.

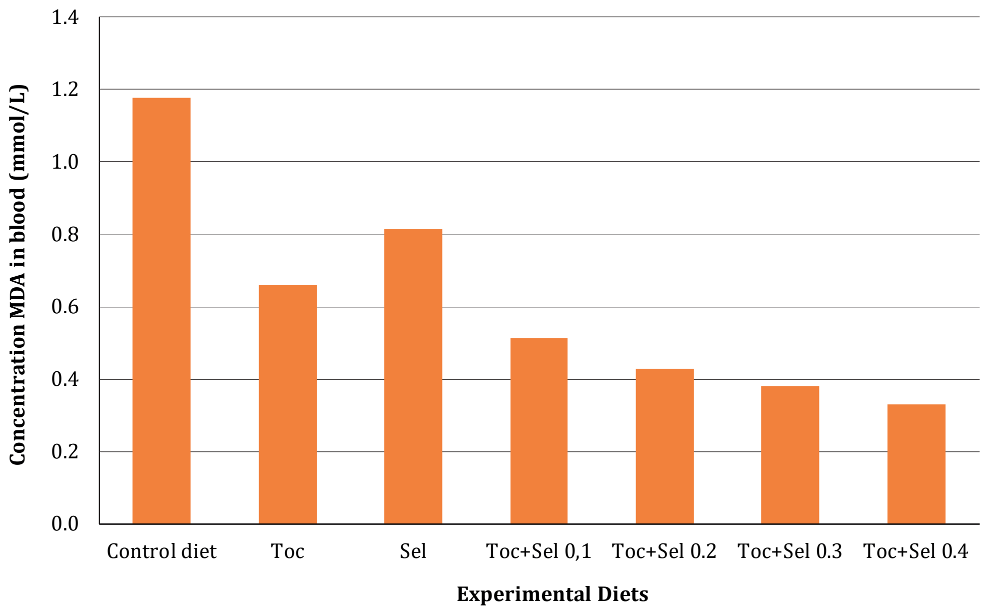

Figure 1. Quail Blood malondialdehyde as a function of vitamin E and Selenium supplementation.

Figura 1. Malondialdehído en sangre de codorniz en función de la suplementación con vitamina E y Selenio.

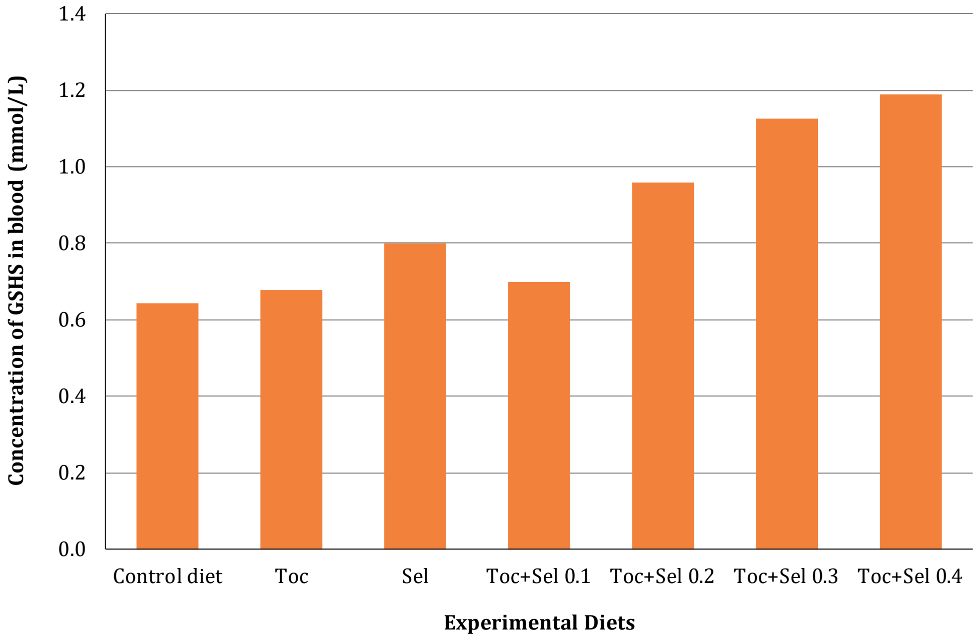

Figure 2. Reduced glutathione peroxidase (GSH-px) in quail blood as a function of vitamin E and Selenium supplementation.

Figura 2. Concentración de Glutatión peroxidasa reducida (GSH-px) en sangre de codorniz en función de la suplementación con vitamina E y Selenio.

Table 5. Malondialdehyde (MDA) in egg yolks of Japanese quails according to storage time.

Tabla 5. Concentración de malondialdehído (MDA) en yemas de huevo de codornices japonesas según el tiempo de almacenamiento.

Experimental diet	Concentration of malondialdehyde/storage time (days)			
	1	8	16	24
Control diet (CD)	53.3 ± 1.6cA	79.9 ± 1.2bB	118.7 ± 7.7dC	232.4 ± 7.8fD
VE diet	18.9 ± 1.8aA	39.1 ± 0.8aB	54.9 ± 3.5bC	69.9 ± 3.5cD
SE diet	33.0 ± 1.7bA	70.1 ± 1.0bB	92.0 ± 6.3cC	116.8 ± 6.4eD
SVE1 diet	19.3 ± 1.9aA	34.0 ± 1.7aB	54.8 ± 4.1bC	84.5 ± 4.9dD
SVE2 diet	20.0 ± 1.8aA	35.0 ± 1.9aB	51.8 ± 2.0bC	58.8 ± 3.2bC
SVE3 diet	19.2 ± 1.9aA	32.7 ± 1.1aB	47.7 ± 3.5bC	55.7 ± 4.0bC
SVE4 diet	19.5 ± 1.9aA	29.8 ± 1.1aB	40.8 ± 2.4aC	47.4 ± 3.1aC
P - value	0.00518	0.0000	0.0000	0.0033

Diets: CD + 200 mg of vitamin E (VE); CD + 0.20 ppm organic Selenium (SE); CD + 0.10 ppm organic Selenium + 200 mg vitamin E (SVE1); CD + 0.20 ppm organic Selenium + 200 mg vitamin E (SVE2); CD + 0.30 ppm of organic Selenium + 200 mg of vitamin E (SVE3); CD + 0.40 ppm organic Selenium + 200 mg vitamin E (SVE4). = Different lower/upper case letters indicate significant differences at p≤ 0.05for Tukey test.

Dietas: CD + 200 mg de vitamina E (VE); CD + 0,20 ppm de Selenio orgánico (SE); CD + 0,10 ppm de Selenio orgánico + 200 mg de vitamina E (SVE1); CD + 0,20 ppm de Selenio orgánico + 200 mg de vitamina E (SVE2); CD + 0,30 ppm de Selenio orgánico + 200 mg de vitamina E (SVE3); CD + 0,40 ppm de Selenio orgánico + 200 mg de vitamina E (SVE4). Las medias seguidas de diferentes letras en columnas o líneas, difieren en el nivel de probabilidad de 0,05 según la prueba de Tukey.

Discussion

These results indicate a possible toxic effect of Selenium (6) on quail folliculogenesis, increasing the production of infertile eggs, as also studied by Oldfield (1987), Spallholz & Hoffman (2002) and Fairbrother *et al.* (2012).

Contrarily, Canogullari *et al.* (2010) reported no significant differences in feed intake, egg yield and egg weight. They explained that organic Selenium supplementation was more effective than inorganic supplementation, at increasing egg Selenium content. According to

Liu *et al.* (2020), adding 0.5 mg/kg of dietary Selenium significantly improved egg production performance of hens, while organic supplementation allowed higher yolk Selenium than inorganic Selenium. These results corroborate those scientific results on nutritional requirements and the interactions of micronutrients with laying quails during their early stages. Our results agreed with Mori *et al.* (2003), who verified the linear relationship between the concentration of tocopheryl acetate supplemented in chicken diets and yolk α- tocopherol content. As evidenced in the present research, Selenium supplementation affected egg α-tocopherol concentration while the interaction with vitamin E significantly increased GSH-px and MDA concentrations (table 4, page 149).

According to Shah *et al*. (2016), the concentration of glutathione peroxidase and superoxide increased when vitamin E reached 100 and 150 mg/kg in the feed. Antioxidant vitamins have an inverse relationship with lipid peroxidation inside the body, resulting in cell damage. Vitamin E is a natural antioxidant with an essential role against lipid peroxidation (9). Jena *et al*. (2013) reported that superoxide dismutase (SOD) and catalase activity increased significantly when broilers were supplemented with vitamin E under heat stress conditions. Previously, Özkan *et al*. (2007) found that at low temperatures (14.5 to 16.8°C), supplementation of vitamin E in combination with Selenium enhanced broiler liver glutathione peroxidase. Behavioral stress produces free radicals and ROS, which can potentially harm the cell membrane by peroxidation of polyunsaturated fatty acids (10).

As for vitamin E as a function of storage time, yolk malondialdehyde also exhibited a significant difference ($p < 0.05$). Adding dietary vitamin E and/or Selenium increased egg shelf life with a synergistic effect, which replied to the increase of organic Selenium (table 5, page 150). Baylan *et al.* (2011) explained that a high level of Selenium -yeast administration to quail diet increased storage time compared to selenite and control groups.

Vitamin E and Selenium supplementation promoted lower yolk MDA concentration over time (table 4, page 149). However, the associated supplementation of alpha-tocopherol with organic Selenium exhibited higher efficiency in inhibiting lipid peroxidation, evidencing, once more, the synergistic effect of micronutrients when compared to isolated supplementation. These results coincide with Surai (2002) and Akil and Piliang (2012), who reported a synergistic advantage of tocopherols and Selenium protection against oxidative stress and prevention of peroxidation of long-chain fatty acids. Surai and Dvorska (2002) evaluated GSH-px, vitamin E and Selenium activity in stored eggs, showing that combining vitamin E and Selenium was more efficient against lipid peroxidation during storage time.

Conclusion

The supplementation of 200 mg of α-tocopherol and 0.30 ppm of organic Selenium decreased feed intake but did not affect other performance parameters. Egg quality was not influenced by dietary vitamin E and/or Selenium. The synergistic effect of including α- tocopherol and organic Selenium on malondialdehyde levels and glutathione activity was statistically significant especially when the mineral supplementation was increased.

Egg shelf life increased with α-tocopherol and increasing levels of organic Selenium, confirmed by the lower production of malondialdehyde.

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Rescue and participatory conservation of Creole goats in the agro-silvopastoral systems of the Mountains of Guerrero, Mexico

Rescate y conservación participativa de las cabras criollas en los sistemas silvo-agropecuarios de la Montaña de Guerrero, México

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Abstract

The objective was to implement a participatory process involving rescue and conservation of Creole goats in agro-silvopastoral systems, as a development strategy for the indigenous and marginalized region of the Mountains of Guerrero (MG), Mexico. The study focused on the caprine agroecosystem, documenting aspects of goat development and identifying caprine areas in 13 municipalities, zoometrically characterizing 680 goats. One hundred and ten goat producers were interviewed for evaluating farmer perception of goat production. Fifty-seven producers were trained in holistic management, and four producers raised 300 goats in outstanding herds. Data were analyzed using social networks, principal component analysis and correspondence analysis. Two goat agroecosystems were identified: 1) agro-silvopastoralism, with crossbreeding of goat populations and, 2) traditional systems, involving grazing of Creole goats on native vegetation. We identified three types of goats: 1) Mixteco mosaic (61%), 2), Pastoreña (31.8%), and 3) Crossbreeds (7.2%), based on bicoastal diameter, chest depth, body length, thoracic perimeter, height at withers, shoulder point width and liveweight. Smallholder goat farmers in the MG preferred Creole goats for their greater productivity and better environmental adaptation.

Keywords
pastoreña goats • Mixteco mosaic goats • outstanding herd • goat areas

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Resumen

El objetivo fue llevar a cabo un proceso participativo de rescate y conservación de caprinos criollos en los sistemas silvoagropecuarios, para incorporarlos a una estrategia de desarrollo de la región indígena y marginada de la Montaña de Guerrero(MG), México. El agroecosistema caprino se estudió para describir la dinámica de desarrollo, identificación de las zonas caprinas en 13 municipios y la caracterización zoométrica de 680 cabras. Se encuestó a 110 productores para evaluar la percepción de la producción de cabras, 57 productores fueron formados en manejo holístico y cuatro productores para la crianza de 300 cabras en rebaños sobresalientes. Los datos se analizaron utilizando redes sociales, componentes principales y correspondencia. Se identificaron dos agroecosistemas caprinos: 1) Agrosilvopastoralismo, con proceso de hibridación alta de las poblaciones de cabras y 2) el tradicional, de pastoreo en vegetación nativa con cabra criolla. Se caracterizaron tres tipos de cabras: 1) mosaico mixteco (61%), 2) cabras pastoreñas (31,8%) y 3) cruzas (7,2%), discriminadas por el diámetro bicostal, profundidad de pecho, longitud del cuerpo, perímetro torácico, alzada a la cruz, distancia entre encuentros y peso vivo. Los productores de caprinos de la MG prefieren las cabras criollas porque son más productivas y mejor adaptadas a las condiciones ambientales.

Palabras clave

cabra pastoreña • mosaico mixteco • rebaño sobresaliente • zonas caprinas

Introduction

Resource diversity in Creole genetics constitutes one major global concern (12). Threats to the Creole goat population include interbreeding with exotic breeds (6, 26), the economic pressure related to traditional breed abandonment towards intensive management systems (41), and the global trend regarding livestock intensification, specialization, and desertion of marginal lands (2).

Conservation programs for Creole goats enable smallholders to prevent Creole breed loss (10, 14, 28). These breeds are adapted to adverse climatic conditions (1), surviving in semi-arid and arid environments (5, 8, 38), and consuming forage grown on marginal lands (27). These capacities have promoted the preservation of pastoral traditions (38), while also promoting sustainability (5, 26, 42). Creole goat raising in rural communities represents a global security system, ensuring a sustainable food source for families (1, 31), and household finance sustains (27, 43), strengthening biodiversity (37) and breed conservation (22, 25, 40).

Studies on Creole goats in certain regions of Mexico have demonstrated several advantages of local genotypes over the improved, recently introduced breeds. Tarahumara goats in Chihuahua´s Mountains (3), local goats in the Comarca Lagunera (24), black goats of Querétaro (4), and pastoreña goats in the Mixteca region (39, 45, 46, 47) exemplify these facts. Farmer-participatory evaluation of exotic goat breeds and their crosses with the native Creole goat breeds of the MG region of Mexico, has not yet been reported. In this sense, the hypothesis stated that by taking advantage of the adaptive capacity and productivity of the MG Creole goats, integrating them into a poor smallholders-participatory program for their rescue and conservation, became possible. The objectives of this study were: 1) to establish farmer-participatory programs to rescue and conserve the Creole goat breed in agro-silvopastoral systems with low resource smallholders; and 2) to incorporate them into a development strategy related to the agro-silvopastoral systems of the indigenous and marginalized region of the MG, Mexico.

Materials and methods

Study site

The study was conducted in the MG region, with a population of about 406,000 inhabitants. The site is part of the "Sierra Madre del Sur", located northeastern of the state of Guerrero, Mexico, at 17°20'25'' and 17°42'29'' North latitude and 98°26'48'' and 98°48'37'' West longitude. Site topography consists of mountains, hills, and inter-montane valleys, ranging from 500 to 3000 meters a.s.l. Site climates include temperate humid and sub-humid, semi-warm sub-humid and humid, and semi-arid warm (18). Annual precipitation varies between 700 and 900 mm. The most common types of soil include Regosol, Litosol, Cambisol, and Rendzinas. The dominant vegetation is low deciduous forest, groves of pine-oak, and pine forest.

This study shows information recorded during the process of rescue and conservation of Creole goats, with farmer participation, from 2012-2021. We recorded data from 36 communities in the municipalities of Alcozauca, Alpoyeca, Cochoapa el Grande, Malinaltepec, Atlixtac, Tlapa de Comonfort, Huamuxtitlán, Xochihuehuetlán, Tlalixtaquilla, Olinalá and Cualác, following the guidelines indicated for in vivo conservation of animal genetic resources (10, 15).

Data collection

Goat agro-ecosystem dynamics were analyzed with the historical information from the MG, compiled since 1979 by the Mountain Guerrero Plan and Mountain Prioritize Attention Micro-region (MAP) of the "Colegio de Postgraduados-Campus Puebla".

Caprine areas in 36 communities throughout the region were identified with the support of extension services and members of farmers organizations. For each community, the following information was recorded: geographical location, predominant type of goats (Creoles, Pastoreña, and crosses), vegetation type for grazing, and the willingness of smallholder goat keepers to participate in the project.

Zoometric and liveweight (LW) characterization of adult goats were undertaken following recommended procedures (13, 19, 21): 1) length (HL) and head width (HW), 2) height at withers (HaW), 3) thoracic perimeter (TP), 4) body length (BL), 5) bicoastal diameter (BD), 6) shoulder point width (SW), 7) chest depth (CD), 8) rump width (RW), and 9) rump length (RL).

Perceptions on goat raising were documented from a sample of 112 households. The variables considered were: 1) breed preference; either Creole or exotic commercial breed, and 2) criteria for selecting goat breeding stock.

Farmers were trained to follow a holistic goat management approach with dialogue of knowledge between farmers and technicians (44), in order to develop more consistent strategies with farmer perception (23, 32). Discussed topics included: 1) feeding (use of pasture farms), 2) goat health, 3) drinking water management, and 4) selection of breeding stock. A group of four farmers was selected for productive, reproductive, economic, and marketing evaluation of the breeding stock of 300 Creole goats.

Data processing

Information from goat areas was analyzed using the Social Network Technique with UCINET (Version 6.730 | June 18, 2021) from Analytic Technologies. Initially, a symmetric matrix of the goat region indicated either the existence of goat tradition (represented by 1) or the loss of goat tradition (represented by 0). In the first matrix, two more matrices were incorporated: Goat genetic type (Pastoreña and Creole goats; Mixteco mosaic and goat crosses), and type of vegetation (deciduous forest, pine-oak forest, and agricultural areas-pasturelands). Results showing the relationship among goat tradition, type of goat and foraging vegetation are presented in figure 1 (page 156).

FactoMineR package (20) from R Programming language (34) was chosen to analyze zoometric variables and live weight data creating a two-dimensional figure with goat population groups. A simple correspondence (ca) procedure (29) revealed criteria related to objective and selection, as applied by farmers.

Results and discussion

Smallholder identification and goat preference

This study revealed that Creole goats predominate in 36.1% of municipalities (figure 1, page 156).

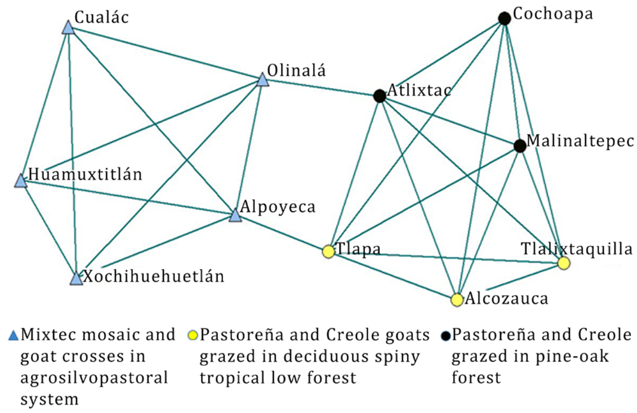


Figure 1. Relationship between caprine tradition, type of goat and vegetation in the municipalities of the MG, Mexico.

Figura 1. Relación de la tradición caprina, tipo genético y vegetación utilizada en las zonas productoras de cabras en la MG, México.

Farmers from Alpoyeca, Huamuxtitlán, Xochihuehuetlán, Olinalá, and Cualác predominantly owned crossbreeds of Creole with exotic commercial breeds (Alpine, Boer and Nubian crosses), grazed in the agro-silvopastoral system. Farmers from Alcozauca, Cochoapa el Grande, Malinaltepec, Tlalixtaquilla, Atlixtac, and Tlapa de Comonfort predominately owned Creole goats, which they grazed on pine-oak and low spiny deciduous forests. They also raised Pastoreña goats, being the most traditional farmers.

Figure 1 presents two goat producing regions and management systems. The left side shows municipalities, where goat crossbreeding is relatively intense, predominantly between Mixteco mosaic and exotic commercial goats (Alpine, Boer and Nubian crosses). The goats graze agricultural (arable land) areas and communal pastures. Government programs that promote exotic commercial goat production with little or no consideration for the opinions and customs of the smallholder farmers have a high impact on these municipalities. The right side shows municipalities where Creole goats predominate (Pastoreña and Creole goats) and where the government program for promoting commercial goat production has had little or no impact. The goats are grazed in the deciduous spiny tropical low forest (Tlalixtaquilla, Alcozauca, and Tlapa de Comonfort) and pine-oak forest (Atlixtac, Malinaltepec, and Cochoapa el Grande), indicating that Creole goats are an invaluable resource for environmental adaptation in the context of climate change (38).

Figure 2 (page 157) presents a correspondence analysis of goat populations as perceived by smallholders. Data indicates that farmers preferred Creole goats. Goat crosses preference (Alpine and Nubian crosses) as selection criteria, stands far from the origin of the axes, indicating lower frequency among interviewed smallholders. This observation is in contrast with the findings for Pakistani Beetal goats (33), where milk and meat production constituted selection criteria. In this study, goat producers indicated that environmental adaptation correlates with meat production among Creole goats, evidencing an increased awareness of their biological qualities, behaviour and environmental adaptability, as stated in Tanzania (30).

Goat body size was the primary selection criterion (figure 3, page 157). Specifically, farmers related body size to height at withers. Tall goats are valued for their ability to move during grazing and browsing in the shrubby vegetation, concurring with previous reports from Pakistan (33).

Secondly, some farmers do not use any specific criteria, especially in the selection of kid goats and at herd level.

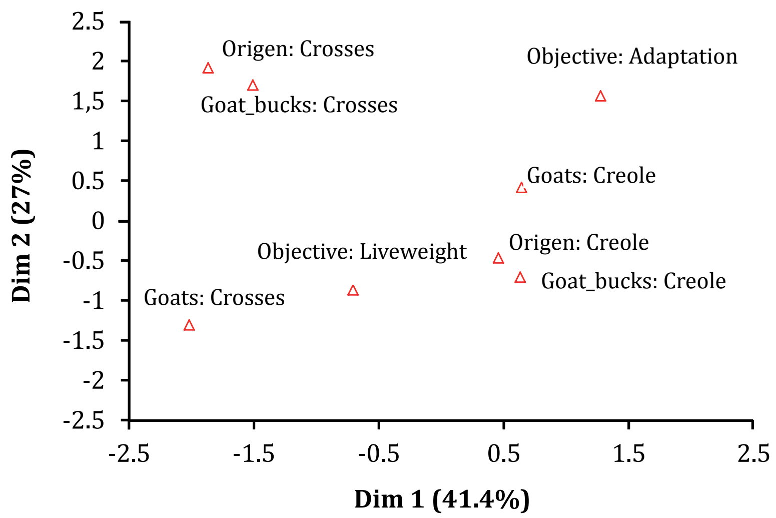


Figure 2. Graphical representation of the correspondence analysis on origin, breed and objective of goat selection in the MG, Mexico.

Figura 2. Representación gráfica del análisis de correspondencia del origen, raza y objetivo de selección de los caprinos señalados por los campesinos en la MG, México.

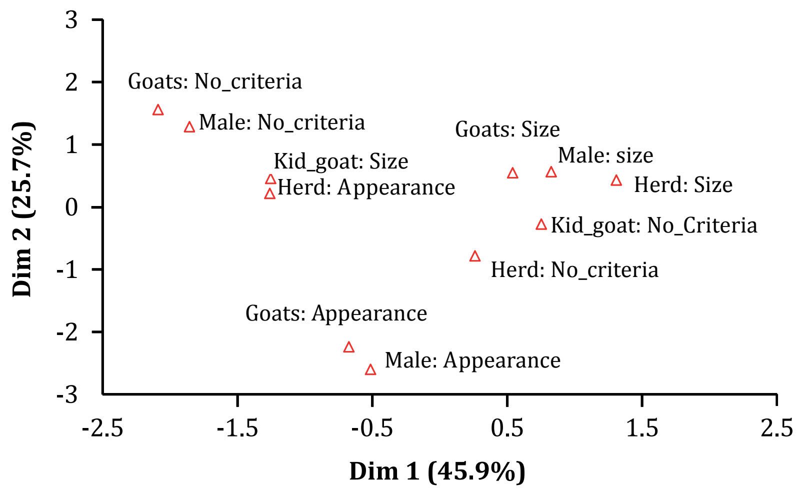
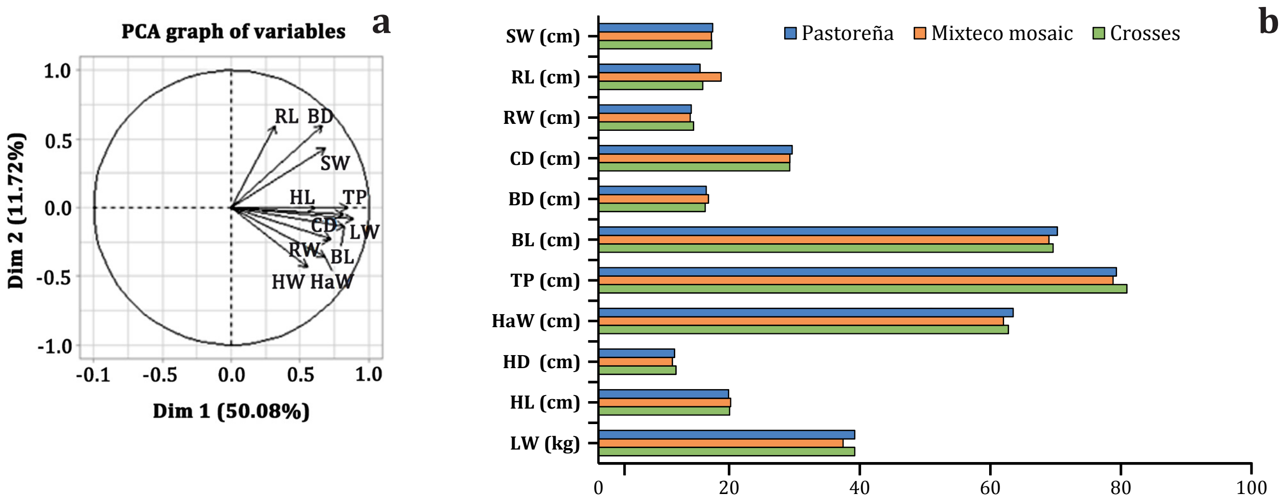


Figure 3. Graphical representation of the correspondence analysis regarding selection criteria in terms of herd, goats, male and goat kids employed by farmers in the MG, Mexico.

Figura 3. Representación gráfica del análisis de correspondencia de los criterios de selección del rebaño, cabras, sementales y crías utilizados por los campesinos en la MG, México.

Creole Goats phenotypic characteristics

Zoometric evaluation of goat populations revealed that farmers breed crosses (7.2%), Pastoreña goats (31.8%), and Mixteco mosaic goats (61%). The correspondence analysis in figure 4a (page 158) shows body traits and liveweight indicating differences between populations, probably corresponding to system diversity as observed among Cuban goats (21). In order of importance: bicoastal diameter, chest depth, body length, thoracic perimeter, height at withers, shoulder point width, and liveweight.



HL: head length, HW: head width, HaW: height at withers, TP: thoracic perimeter, BL: body length, BD: bicoastal diameter, SW: shoulder point width, CD: chest depth, RW: rump width, RL: rump length.

HL: longitud de cabeza, HW: ancho de cabeza, HaW: altura a la cruz, TP: perímetro torácico, BL: longitud del cuerpo, BD: diámetro bicostal, SW: anchura de pecho, CD: profundidad del pecho, RW: ancho de grupa, RL: longitud de grupa.

Figure 4. Variation in zoometric body traits and liveweight (LW) among goat population in the MG, Mexico.
Figura 4. Variabilidad de las medidas zoométricas y peso vivo (PL) de las poblaciones de cabras en la MG, México.

Breed profile and conformation of goats from the MG are presented in figure 4b. Pastoreña goat showed the greatest body length, height at withers and liveweight; characteristics associated with meat production. Goat height relates to adaptation and transhumance travel ability. The commercial genetic type influenced crossbreed and Mixteco mosaic goats with a greater thoracic perimeter, rump length and width, and less height at withers. The presence of commercial goats in the region indicates a need for an urgent conservation program for Creole goats, similar to that implemented in other areas of America (17, 42), Africa (27), and Italy (7, 9).

The principal component analysis (PCA) showed overlapping goat populations in a two-dimensional space (figure 5, page 159), indicating a slight difference in zoometric measurements and liveweight among the populations. Smallholders know that Creole goats are superior to exotic commercial goats, partly because they are better adapted to marginal areas, where forage is scarce (35). Herds of Pastoreña goats are uniform in terms of their morpho-structural characteristics and liveweight, key factors associated with natural selection for meat production over several centuries (11, 16, 36, 46).

Holistic training

Using dialogue of knowledge, the following training topics were identified for rescuing these goats: health-environment, transboundary goat diseases resulting from the introduction of exotic commercial goats, disease prevention and treatment, nutritional blocks, breeding stock supplementation, identification, and selection of Creole goats. In addition, smallholders helped with data collection in order to identify goat populations, evaluate productivity (adult live weight, kid daily weight gain, and body condition), and identify unproductive goats to reduce pressure on pastureland. Dialogue of knowledge allowed innovation of the traditional goat production systems in the region, reducing smallholders resistance, who did not show any inclination towards modern knowledge (48).

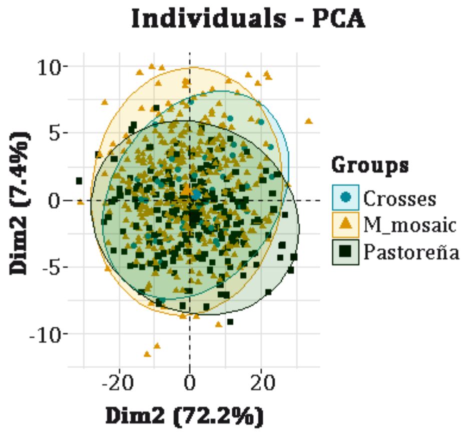


Figure 5. Scatter plot showing goat population breeds according to zoometric variables and liveweight in the MG, México. M_mosaic, Mixteco mosaic goats.

Figura 5. Diagrama de dispersión de las razas de cabras según variables zoométricas y peso vivo en la MG, México. M_mosaic, cabras mosaico Mixteco.

Rescue process

Figure 6 shows the participatory management of Creole goats, as part of the rescue process. We categorized four smallholders as participants with outstanding herds. Three hundred goats raised in outstanding herds were selected as goats and bucks with above-average zoometric measurements and liveweight, grazed on shrubby vegetation of low deciduous forest, free from brucella, white or bay in colour and spiral horns; typical of the Pastoreña goat. Likewise, Creole goats from outstanding herds manifested the least weight loss in the annual dry season, which improved goat selling price, and increasing family income. In this study, producers classified Creole goats as part of their cultural tradition, as a means to preserve cultural heritage (28).

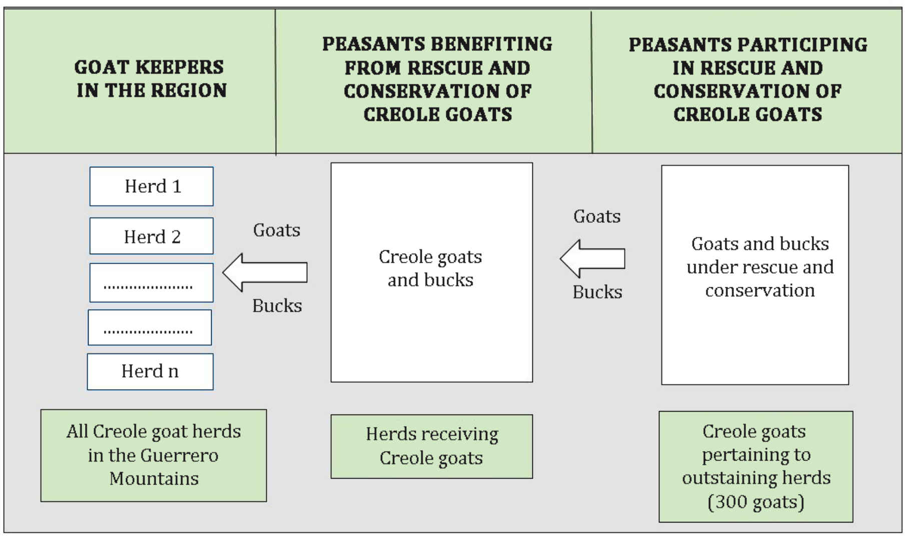


Figure 6. Rescue and conservation processes for Creole goats by peasants in the MG, México.

Figura 6. Proceso de rescate y conservación de los caprinos criollos por los campesinos en la MG, México.

Conclusion

This preliminary study on goat production systems in the Mexican MG showed two production systems, namely: agro-silvopastoralism with crosses between Creole and exotic goats (Nubian, Boer and Alpine crosses), and traditional production, involving grazing of Creole goats on native vegetation. Crossbreeding of Pastoreña and Creole with commercial exotic breeds represents the main threat to the sustainable farming of Creole goats in the MG. Producers cite both adaptation and body size as selection criteria promoting Creole goat maintenance. Pastoreña goats are considered the best meat producers in the MG, due to a greater body length, height at the withers and live weight, and grazing ability in mountain areas. Producers used traditional knowledge and low-input technology for the rescue and *in situ* conservation of Creole goats in the MG, through an assisted holistic management. Government agencies must consider participative planning to rescue Creole goat populations, if traditional goat production and breeding systems in marginalized areas, are to continue.

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Indole-3-butyric acid, an alternative to GA_3 for bunch quality enhancing of table grape *Vitis vinifera* L. cv. Superior Seedless

Ácido indol-3-butírico, una alternativa al uso de GA_3 para incrementar la calidad de racimos en uva de mesa *Vitis vinifera* L. cv. Superior Seedless

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Abstract

Gibberellic acid (GA_3) is the most widely used plant growth regulator to thin out bunches and increase berry size in seedless table grapes, but there is evidence of its negative effects, including loss of fertility and malformations of the rachis. This study analyses the effects of applying the auxin indole-3-butyric acid (IBA), as a novel alternative, on fruit yield, bunch structure, anatomy and postharvest quality of cv. Superior Seedless. The application of IBA and GA_3 at different phenological stages (15 cm shoot length, full bloom, fruit set and pea-size berry) and doses (2, 20 and 50 ppm) were compared in a two-growing season experiment. Spraying IBA at full bloom or fruit set, improved bunch weight by increasing the size and weight of the berries, correlating with the promotion of rachis vascular tissues. Bunches treated with IBA retained a greater number of berries at harvest without generating compactness, since the elongation of the rachis internodes and lateral shoulders were also promoted. In addition, IBA augmented postharvest quality of bunches by reducing rachis browning and increasing berry firmness. These results suggest that the use of IBA is a beneficial technology to improve bunch structure and quality in seedless grapes.

Keywords

auxin • gibberellins • plant hormones • bunch architecture • seedless table grapes

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Resumen

El ácido giberélico (GA_3) es el regulador de crecimiento vegetal más utilizado para ralear flores en racimos y aumentar el tamaño de las bayas en uvas de mesa sin semilla, pero hay evidencia de efectos negativos por su uso, incluyendo pérdida de fertilidad y malformaciones del raquis. En este estudio se analizan los efectos de aplicar la auxina ácida indol-3-butírico (IBA), como alternativa novedosa, sobre el rendimiento de frutos, estructura del racimo, anatomía y calidad poscosecha del cv. Superior Seedless. Se comparó la aplicación de IBA y GA_3 en diferentes estadios fenológicos (brote de 15 cm, floración plena, cuaje y baya grano arveja) y dosis (2, 20 y 50 ppm), en un experimento de dos temporadas de cultivo. La aspersión de IBA en floración plena o en cuaje mejoró el peso del racimo al aumentar el tamaño y el peso de las bayas, correlacionándose con la promoción de los tejidos vasculares del raquis. Los racimos tratados con IBA retuvieron un mayor número de bayas a cosecha sin generar compacidad, ya que también se favoreció el alargamiento de los entrenudos del raquis y la longitud de los laterales. Además, IBA aumentó la calidad poscosecha de los racimos al reducir el oscurecimiento del raquis y aumentar la firmeza de la baya. Estos resultados sugieren que el uso de IBA es una tecnología beneficiosa para mejorar la estructura y calidad de los racimos en uvas sin semillas.

Palabras clave

auxina • giberelinas • hormonas vegetales • arquitectura de racimos • uva de mesa sin semilla

Introduction

Table grape quality influences consumer decision and sale prices. This quality is related to bunch architecture, berry size and firmness. The quality of cultivars that naturally set straggly bunches can be improved by physical and chemical manipulations with plant growth regulators (PGR; 10). Bunch compactness is modified by PGRs, affecting number and size of berries and their spatial arrangement through rachis architecture (32). In the table grape industry, gibberellic acid (GA_3) is one of the most used PGR for bunch thinning, decreasing berry set and increasing berry size (9, 24, 29), especially in seedless table grape cultivars that naturally set compact bunches with small berries.

GA_3 is used alone or mixed with other PGRs such as cytokinins and auxins (31). However, not all the effects reported for GA_3 are beneficial, since it can decrease (9, 22) and inhibit (24) bud fruitfulness in the season following its application. Reduction in berry skin coloration and increased occurrence of bunches with berries that differ greatly in size and maturity at harvest, known as "millerandage", are reported among GA_3 detrimental effects (2, 9). In addition, GA_3 applications have other adverse effects, such as increased incidence of berry drop (15). Another PGR widely used to increase bunch quality in table grapes is CPPU (Forchlorfenuron; 34), and although it is not synthesized by plants, it is a cytokinin that mimics GA_3 and produces positive results without affecting bud fertility. An additional tool for table grapes production is the use of biostimulants, products of diverse biological origin containing a mixture of PGRs, but with incomplete identified composition of microbial species that produce PGRs or regulate their content in plant tissues (3, 28). In Argentina, these products are registered but no specification regarding their use on vines is available (https://www.argentina.gob.ar/produccion-organica/listado/oficial-de-insumos-comerciales).

In search for an alternative to GA_3, a preliminary study compared the effects of PGRs synthesized by plants on bunch quality and crop yield using GA_3, 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and abscisic acid (ABA) on *Vitis vinifera* L. cv. Superior Seedless. The results of this experiment showed a positive effect on bunch quality with IBA application (tables S1, S2, S3). Therefore, further experiments were performed to evaluate the performance of IBA on bunch quality as an alternative to GA_3.

Normally, the initial stimulus for fruit growth begins with pollination and correlates with elevated levels of auxins, mainly indole-3-acetic acid (IAA; 4). After fertilization, fruit growth depends on auxins produced in developing seeds, through the endosperm at the initial stages and the developing embryo during the later stages (30). Coombe (1960) evaluated changes in auxins during fruit development (from anthesis to maturity) on seeded

(Muscat, Emperor) and seedless (Sultanina, Emperor Seedless and Corinth) grape cultivars. In general, auxins rise from low levels after anthesis to high levels a few weeks later, and are maintained for long periods in seeded cultivars. Costantini *et al.* (2007) demonstrated that auxin signal transduction allows fruit set in the absence of pollination (parthenocarpy), and that auxins enhance fertility. In addition, auxins strongly modulate growth (17) via cell division, cell expansion (13) and vascular development (1, 21).

The main auxin in higher plants is IAA, which is synthesized in meristems, young leaves and developing fruit and seeds, but there are other natural molecules with auxin activity. IBA is a natural auxin synthesized by higher plants, commonly used for rooting and micro propagation (12). It has also been shown as more effective than IAA in lateral root formation (14), being considerably less expensive. At present, few publications have reported the effect of IBA on grape bunch quality and yield, and these studies have been performed with PGRs blends containing IBA, that is mixed with cytokinins, gibberellins and auxins (31). Against the above framework, the hypothesis is that IBA sprayed to commercial vineyards improves fruit set, the number of berries per bunch, fruit growth, bunch quality and consequently yield in table grape.

Superior Seedless, also known as Sugraone, is one of the main white seedless table grapes worldwide and the most exported from the Province of San Juan, Argentina, with a cultivated area of 2359.6 ha (18). It usually has very low fruitfulness (26), with stenospermocarpic berries (*i.e.* the embryos begin their development after fertilization but abort at an early developmental stage, leaving a seed trace; 5), implying that the cause relies on the fact that one of the main auxin sources, the embryo, is absent.

The aim of this study was to examine exogenously applied IBA as an alternative tool to improve seedless table grapes yield and quality indicators. The effect of IBA, applied at different dosages and phenological stages, was evaluated in comparison with GA_3 on Superior Seedless bunch structure, yield and quality. Furthermore, an anatomical analysis was performed to explain PGRs effects on bunch architecture. After the first preliminary experiment and with IBA showing the best results, a second growing season evaluated and determined the most effective dose and adequate phenological stage.

Material and methods

Plant material and treatments

The trial was conducted over two seasons: 2012-2013 and 2014-2015 in a commercial *Vitis vinifera* L. cv. Superior Seedless vineyard, located at Carpintería, San Juan, Argentina (31°43'S and 68°35'W; 642 m a.s.l.). Grapevines were planted in 2005, own rooted, spaced at 2.5 x 2.5 m, trained on a local trellis system called "Parral "or overhead. The soil of the experimental site was sandy with 60-80% stone content. The vineyard was drip irrigated. Vines were cane pruned with 10 canes per vine and 12 buds each. When inflorescences were developed [phenological stage 13 (6)], vines were thinned to 30 bunches per plant. Only well-developed basal clusters were left. A completely randomized design with 5 replicates, with one vine as experimental unit, was selected according to vigor homogeneity (trunk diameter). During 2012-2013, three doses of IBA and GA_3 (2, 20 and 50 ppm) were separately sprayed at three phenological stages (6); 15 cm shoot length (Stage 13), full bloom (Stage 25-26) and berries pea size (Stage 31). Commercial IBA (SIGEL, GEO SRL, Argentina) and GA_3 (10%, GIBERELINA KA, S. Ando & Cía. SA, Argentina) were dissolved in water, with 0.1% (v/v) of Triton X-100 as surfactant and a minimum volume of 96% aqueous ethanol. A solution containing water with the concentration of surfactant and ethanol as described above was used as control. Treatments were applied with a backpack sprayer to the whole vine until runoff (*ca*. 100 mL $plant^{-1}$). Applications were done in the late afternoon to avoid photo degradation and to prevent the solution from fast drying off by high temperatures. In the second season, three IBA doses (2, 20 and 50 ppm) and 4 phenological stages, 15 cm shoot length (Stage 13), full bloom (Stage 25-26), fruit set (Stage 26-27) and berries pea size (Stage 31) were evaluated.

Yield components

Four bunches per plant were selected and used as observational units (20 bunches per treatment). After berry softening phase (Stage 36, Coombe 1995), fruit ripening was weekly monitored by measuring accumulation of total soluble solids (TSS) with a hand-held refractometer (Pocket PAL-1, Atago®, Tokyo, Japan). All the treatments were harvested when the control treatment reached 14 to 15°Brix. The collected bunches per experimental unit were placed in plastic bags and kept in ice to prevent dehydration. Bunch fresh weight (FW), berry FW and berry size (diameter and length) from 20 randomly selected berries per bunch were determined. Berry number was calculated based on bunch and berry weights.

Bunch architecture and Anatomical analysis

Rachis FW was registered immediately after berry removal. After that, bunch structure was evaluated by measuring length and diameter of the central rachis, lateral rachis ramifications (4 upper lateral) and pedicels (at the insertion point of the berry) from 6 randomly selected pedicels. All diameters were measured with a digital caliper. In the second season bunch compactness was assessed by ascertaining the distance between rachis laterals (internodes) and berries number per cm of 4 upper lateral ramifications (figure 1).

Rachis length and diameter (Ø Rachis), lateral rachis length and diameter (Ø Lateral), pedicel length and diameter (Ø Pedicel), bunch rachis lateral distance (Internode) and number of berries per cm of upper lateral rachis (#Berries cm^{-1}).

Longitud y diámetro de raquis (Ø Rachis), longitud y diámetro de raquis laterales (Ø Lateral), longitud y diámetro de pedicelos (Ø Pedicel), distancia entre raquis secundarios (internado) y número de bayas por cm en raquis laterales superiores (#Berries cm^{-1}).

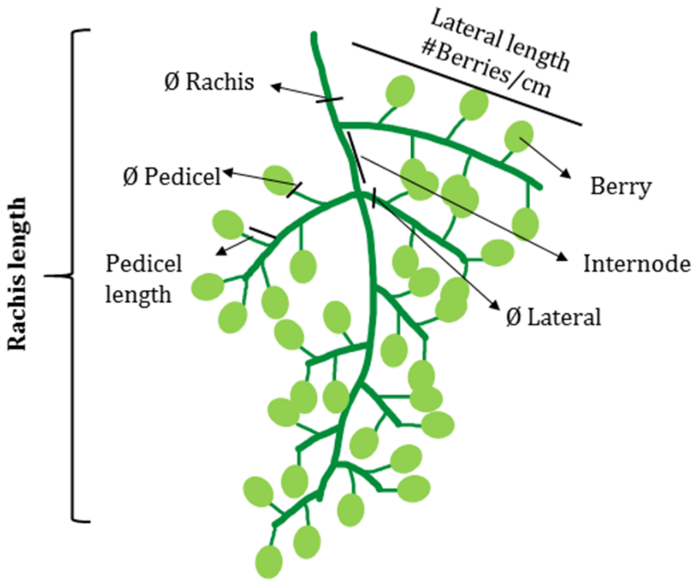

Figure 1. Schematic representation of bunch architecture.

Figura 1. Esquema de la arquitectura de racimos.

Samples for anatomical observations were collected from bunches treated at full bloom with IBA 20 ppm, GA_3 20 ppm and control plants. Histological micro-sections (13 μm) of the rachis (2 cm above the upper lateral rachis) were prepared using a rotary standard microtome, stained, and evaluated with a microscope (Model 16, Carl Zeiss, Gottingen, Germany). Photomicrographs were taken and digitalized with a camera (AxioCamHRc, Carl Zeiss) according to the protocol of Travaglia *et al.* (2012). Xylem and phloem areas were calculated using the software Image Pro-Plus (Media Cibernetics Inc, Rockville, MD).

Postharvest quality indicators

After harvest, 4 bunches per experimental unit were packed. Each bunch was individually bagged in perforated polyethylene and packed in carton boxes with a SO_2 generator pad (fast and slow-release phases; 1 g Kg^{-1} of fruit) to prevent decay. The packages were air cooled to 4 °C for 9 hours. Then, these packed bunches were cold stored for 60 days at 0°C and 95% relative humidity for post-harvest evaluation. Rachis browning, berry shatter and berry firmness were evaluated after cold storage. The rachis of each bunch was visually evaluated with a 1-4 scale where: 1 = healthy, entire rachis including pedicels are green and healthy; 2 = slight, rachis in good condition, but pedicel browning is noticeable; 3 = moderate, browning is visible in pedicels and secondary rachis; 4 = severe, pedicels, secondary and primary rachis are completely brown (8). Percentage of berry shattering (loss of berries from the cap stem) was calculated based on detached berries and total number of berries per bunch. Berry firmness was measured on the equatorial position of 20 randomly selected berries per bunch with a penetrometer (Fruit Pressure Tester, Model FT 327, Italy).

Statistical analysis

The analyses were performed with InfoStat Software (InfoStat version 2009; Grupo InfoStat, Córdoba, Argentina) using generalized linear models. Means were separated using LSD Fisher test with a level of significance $P \leq 0.05$. Principal component analysis (PCA) was performed using biplot graphics and standardized (centered and variance-scaled) data (InfoStat version 2009; Grupo InfoStat, Córdoba, Argentina).

Results

Yield components

During season 2012-2013, bunch FW, berry FW, berry diameter and berry length were increased in almost all IBA and GA_3 treatments as compared to control (table 1, page 168). The most prominent effect on bunch FW was obtained with IBA applied at full bloom, in a dosage dependent manner and with an increase of up to 84% as compared to the control. Furthermore, berry FW with IBA at full bloom, increased 37 to 50% more than the control. The number of berries per bunch decreased by 33% under GA_3 50 ppm and increased by 21 to 28% at all doses of IBA, both applied at full bloom.

Treatments did not affect TSS, except for GA_3 50 ppm at full bloom, which showed higher TSS than any other treatment.

In the 2014-2015 season, bunch FW, berry FW and berry size were increased by IBA treatments as compared to control (table 1, page 168). However, during the second season, IBA applied at fruit set produced the highest bunch FW and number of berries per bunch, increasing 60% and 30 to 46% respectively, with respect to control (table 1, page 168). In addition, this treatment did not delay ripening, which achieved the same TSS concentration as the control. Even though the treatments IBA 2 and 50 ppm at shoot 15 cm, IBA at full bloom (all doses), and IBA 50 ppm at berries pea size showed significant difference with IBA at fruit set and the control, all treatments achieved commercial maturity (14 to 15 °Brix) (table 1, page 168).

In both seasons, all treatments increased berry diameter and length, by 1 to 2 mm and 3 to 4 mm respectively, compared to control, without affecting the typical oval shape of Superior Seedless berries (berry diameter to length berry ratio; table 1, page 168).

A seasonal effect was also observed, *e.g.* during 2014-2015, bunch FW and berry FW in control were 24% lower than in 2012-2013; however, differences among the treatments with PGRs still occurred, and similar trends between treatments and control could be observed.

Bunch architecture and Anatomical analysis

Table 2 (page 169) indicates PGR effects on bunch structure. During the 2012-2013 season, the highest rachis elongations were observed with GA_3 at 50 ppm applied at 15 cm shoot length. However, this treatment showed twisted rachis (overdose symptoms; figure 1S). An increase in pedicel diameter occurred with 2 ppm GA_3 application at 15 cm shoot length, full bloom (all doses), and berry pea size (2 and 50 ppm). All the treatments with PGRs resulted in an increase in pedicel length. IBA at full bloom (all doses) showed the highest lateral length, the highest rachis diameter and rachis FW.

In the 2014-2015 season, all the IBA treatment increased the rachis FW, lateral rachis length, rachis diameter and the distance between lateral ramifications (rachis internode). In addition, IBA treatments, except for the lower dosages at berry pea size, achieved the highest lateral rachis diameter. The number of berries per cm was increased by IBA at 15 cm shoot length (20 and 50 ppm), and fruit set (50 ppm). Pedicel diameters were not affected by the treatments (table 2, page 169).

Table 1. Yield components at harvest. / **Tabla 1.** Componentes del rendimiento a cosecha.

PGR	Stage	Dose (ppm)	2012/2013 Season							2014/2015 Season						
			Bunch FW (g)	Berry FW (g)	# Berries/ bunch	Ø Berry (mm) diam.	Ø Berry (mm) length	Ø Berry (mm) ratio L/D	TSS (°Brix)	Bunch FW (g)	Berry FW (g)	# Berries/ bunch	Ø Berry (mm) diam.	Ø Berry (mm) length	Ø Berry (mm) ratio L/D	TSS (°Brix)
GA_3	Shoot 15 cm	2	810.30*	6.04*	125.13	19.30*	24.30*	1.33*	15.03	-	-	-	-	-	-	-
		20	768.50*	5.48*	124.74	18.48*	23.25*	1.27*	15.50	-	-	-	-	-	-	-
		50	530.82	5.37*	105.77	18.12*	24.27*	1.27*	15.33	-	-	-	-	-	-	-
	Full bloom	2	460.55	4.70	104.78	18.90*	23.50*	1.27*	15.00	-	-	-	-	-	-	-
		20	678.00	5.62*	92.43	19.06*	24.69*	1.30*	15.57	-	-	-	-	-	-	-
		50	426.75	6.21*	73.76*	18.53*	23.76*	1.33*	16.00*	-	-	-	-	-	-	-
	Berries pea size	2	740.13*	6.63*	114.14	19.66*	23.03*	1.23*	14.90	-	-	-	-	-	-	-
		20	585.88	5.82*	97.58	19.05*	23.94*	1.25*	14.97	-	-	-	-	-	-	-
		50	793.00*	6.69*	116.41	19.42*	25.28*	1.30*	14.90	-	-	-	-	-	-	-
IBA	Shoot 15 cm	2	693.00	6.51*	122.66	19.32*	23.98*	1.23*	14.95	499.29*	4.47*	105.69	18.04*	20.10*	1.13	14.38*
		20	851.00*	6.40*	120.71	19.27*	24.65*	1.27*	14.88	543.17*	4.24*	120.49	17.97*	20.43*	1.14	14.60
		50	872.63*	6.40*	130.91	19.08*	20.88*	1.25*	14.90	599.17*	4.31*	126.63*	17.92*	20.21*	1.15	14.06*
	Full bloom	2	802.42*	6.68*	136.32*	19.48*	24.93*	1.27*	14.90	556.17*	4.19*	123.79	17.58*	19.90*	1.17	14.18*
		20	905.88*	6.08*	141.53*	19.03*	23.52*	1.24*	15.56	485.83*	4.28*	112.56	17.62*	20.39*	1.16	14.30*
		50	938.13*	6.25*	137.34*	19.08*	21.11*	1.29*	14.50	525.00*	4.45*	134.63*	17.83*	21.17*	1.18	14.23*
	Fruit set	2	-	-	-	-	-	-	-	637.17**	4.47*	133.99*	17.78*	20.59*	1.16	15.19
		20	-	-	-	-	-	-	-	605.67*	4.06*	131.17*	17.29*	20.29*	1.18	15.23
		50	-	-	-	-	-	-	-	614.50*	4.48*	146.67**	17.40*	20.99*	1.18	15.02
	Berries pea size	2	620.00	4.89	122.43	18.68*	23.77*	1.17	15.65	461.17*	3.96	107.85	17.64*	20.02*	1.14	15.05
		20	656.00	4.86	124.46	18.16*	24.14*	1.18	15.80	496.17*	4.77*	101.69	18.86*	21.72*	1.16	14.91
		50	890.88*	5.85*	130.82	18.69*	23.90*	1.28*	14.50	528.67*	4.30*	118.36	17.76*	20.66*	1.16	14.24*
Control			510.00	4.44	110.30	17.24	20.14	1.17	15.01	381.17	3.62	100.52	16.68	18.96*	1.14	15.12
ANOVA P(value)			<0.0001	<0.0001	0.0164	0.0038	0.0004	0.0004	0.05	<0.0001	0.0015	0.003	<0.0001	0.0021	0.3253	<0.0001

Punch fresh weight (FW), berry FW, number of berries per bunch (#Berries/bunch), berry diameter (Ø Berry) and length, berry diameter and length ratio (ratio L/D) and berry total soluble solids (TSS) of *Vitis vinifera* L. cv. Superior Seedless plants treated with IBA and GA_3 *vs.* controls, seasons 2012/2013 and 2014/2015. Applications at different phenological stages and doses were evaluated. Values are means of 5 replicates and those followed by an asterisk are significantly different than control (Fisher´s LSD test; P≤0.05).

Peso fresco de racimo (FW), peso fresco de bayas, número de bayas por racimo (#Berries/bunch), diámetro (Ø Berry) y longitud de bayas, relación diámetro y longitud de bayas (ratio L/D) y sólidos solubles totales en baya en plantas de *Vitis vinifera* L. cv. Superior Seedless tratadas con IBA y GA_3 en comparación con el control, temporadas 2012/2013 y 2014/2015. Se evaluaron aplicaciones en diferentes estados fenológicos y dosis. Los valores son la media de 5 repeticiones y los asteriscos indican diferencias significativas en comparación con el control (Test LSD de Fisher; P≤0,05).

Table 2. Bunch structure at harvest. / **Tabla 2.** Estructura del racimo a cosecha.

PGR	Stage	Dose (ppm)	2012/2013 Season Rachis FW (g)	Rachis length (cm)	Lateral Length (cm)	Pedicel length (cm)	Ø Rachis (mm)	Ø Pedicel (mm)	2014/2015 Season Rachis FW (g)	Lateral Length (cm)	Ø Rachis (mm)	Ø Pedicel (mm)	Ø Lateral (mm)	Internode (cm)	# Berries/ cm
GA_3	Shoot 15 cm	2	14.06	30.65	8.68	0.91*	4.91	1.34*	-	-	-	-	-	-	-
		20	12.50	34.28	7.33	0.86*	4.65	0.92	-	-	-	-	-	-	-
		50	11.84	38.71*	11.12*	1.03*	4.43	0.99	-	-	-	-	-	-	-
	Full bloom	2	12.14	32.78	7.43	0.88*	4.56	1.17*	-	-	-	-	-	-	-
		20	10.42	31.42	10.19	0.93*	5.13	1.46*	-	-	-	-	-	-	-
		50	9.83	26.70*	9.30	0.84*	5.22	1.39*	-	-	-	-	-	-	-
	Berries pea size	2	8.63	32.48	9.94	0.89*	4.73	1.31*	-	-	-	-	-	-	-
		20	8.50	28.13	7.80	0.83*	4.36	1.05	-	-	-	-	-	-	-
		50	12.13	31.13	10.33	0.93*	4.64	1.23*	-	-	-	-	-	-	-
IBA	Shoot 15 cm	2	11.41	29.42	9.91	0.86*	4.97	1.09	12.77*	9.72*	6.08*	0.86	2.91*	1.29*	1.74
		20	11.58	27.93	10.23	0.83*	4.29	1.00	13.94*	10.56*	5.69*	0.86	3.07*	1.23*	2.03*
		50	13.17	29.48	10.48	0.83*	4.59	1.02	14.71*	10.82*	5.78*	0.89	2.80*	1.26*	2.08*
	Full bloom	2	15.75*	29.33	11.19*	0.86*	5.36*	1.11	11.54*	10.18*	5.90*	0.94	2.81*	1.26*	1.74
		20	15.17*	31.37	11.09*	0.81*	5.46*	0.87	12.38*	10.79*	6.09*	0.93	3.07*	1.09*	1.58
		50	16.63**	32.03	11.28*	0.85*	4.86	1.22*	13.03*	10.90*	6.34**	0.86	2.98*	1.20*	1.68
	Fruit set	2	-	-	-	-	-	-	13.95*	9.70*	5.48*	0.85	2.86*	1.26*	1.79
		20	-	-	-	-	-	-	13.57*	10.43*	5.97*	0.80	2.85*	1.24*	1.68
		50	-	-	-	-	-	-	13.69*	10.33*	5.68*	0.86	3.09*	1.40*	2.11*
	Berries pea size	2	12.38	31.48	11.01	0.76	6.00**	1.13	11.19*	9.25*	5.91*	0.88	2.48	1.32*	1.68
		20	13.17	30.93	10.58	0.84*	5.65*	1.18*	11.77*	9.38*	5.83*	0.83	2.62*	1.05*	1.72
		50	14.42*	31.63	11.62*	0.84*	4.44	1.12	10.89*	10.71*	6.29*	0.88	3.07*	1.29*	1.75
Control			11.17	30.75	9.42	0.71	4.82	0.92	8.26	8.12	4.50	0.83	2.46	0.89	1.76
ANOVA P(value)			0.0008	<0.0001	0.0007	<0.0001	0.0047	0.0001	0.033	0.0207	<0.0001	0.63	<0.0001	<0.0001	<0.0001

Fresh weight (FW), rachis length and diameter (Ø Rachis), lateral length and lateral diameter (Ø Lateral), pedicel length and pedicel diameter (Ø Pedicel), bunch rachis laterals distance (Lateral distance) and number of berries per cm of upper lateral rachis (#Berries/cm) of *Vitis vinifera* L. cv. Superior Seedless plants treated with GA_3 and IBA vs. control, seasons 2012/2013 and 2014/2015. PGRs were applied at different phenological stages and doses. Values are means (n=5) and asterisk mean that are significantly different than control (Fisher´s LSD test; P≤0.05).

Peso fresco de raquis (FW), longitud y diámetro de raquis (Ø Rachis), longitud y diámetro de raquis laterales (Ø Lateral), longitud y diámetro de pedicelos (Ø Pedicel), distancia entre raquis secundarios (Lateral distance) y número de bayas por cm en raquis laterales superiores (#Berries/cm) en plantas de *Vitis vinifera* L. cv. Superior Seedless tratadas con IBA y GA_3 en comparación con el control, temporada 2012/2013 y 2014/2015. Se evaluaron aplicaciones en diferentes estados fenológicos y dosis. Los valores son medias (n=5) y los asteriscos indican diferencias significativas en comparación con el control (Test LSD de Fisher; P≤0,05).

The anatomical analysis showed that the application of IBA increased the vascular areas in the basal section with respect to the control and GA_3 treatments. In particular, the xylem and phloem areas were increased by IBA 20 ppm applied at full bloom, 59% and 27% respectively, as compared to the control (figure 2). Phloem area showed larger than xylem area, especially in the control, with a reduced xylem to phloem ratio (figure 2).

The PCA analysis shows that the xylem and the phloem areas, bunch FW, berry FW, berry size and the number of berries per bunch were associated with IBA (all doses); markedly for IBA 20 ppm at full bloom (figure 3, page 171).

Samples were prepared using a rotary standard microtome, stained and evaluated with a microscope (Model 16, Carl Zeiss, Gottingen, Germany), and photomicrographs were taken and digitalized with a camera (AxioCamHRc, Carl Zeiss). **A:** Microphotographs of rachis histological anatomy, scale bar 400 µm. X: xylem, Ph: phloem, p: pith, C: cortex. **B:** Total vascular, xylem and phloem area (mm^2 per vascular bundle). Asterisks denote significant differences from the control according to Fisher´s LSD test (*, P≤0.05; ***, P≤0.001).

Las muestras se prepararon utilizando un micrótomo estándar rotatorio, se tiñeron y evaluaron con un microscopio (Modelo 16, Carl Zeiss, Gottingen, Alemania), tomando y digitalizando microfotografías con una cámara (AxioCamHRc, Carl Zeiss). **A**: Microfotografías de la anatomía histológica del raquis, barra de escala 400 µm. X: xilema, Ph: floema, p: médula, C: corteza. **B**: Área vascular total, xilema y floema (mm^2 por haz vascular). Los asteriscos indican diferencias significativas con respecto al control según la prueba LSD de Fisher (*, P≤0,05; ***, P≤0,001).

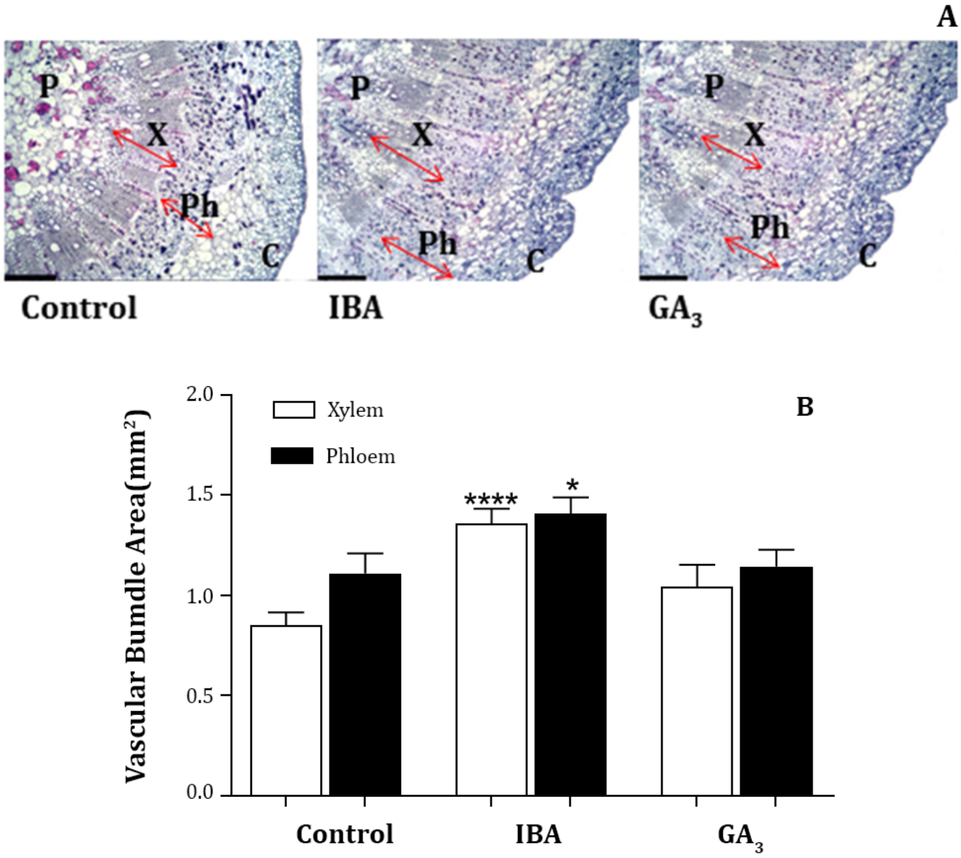

Figure 2. Across-sections (13 µm) of the rachis, 2 cm above the upper lateral rachis, of *Vitis vinifera* L. cv. Superior Seedless plants at harvest treated with IBA 20 ppm and GA_3 20 ppm at full bloom stage and control.

Figura 2. Secciones transversales (13 µm) del raquis, 2 cm por encima del raquis lateral superior en cosecha, en plantas de *Vitis vinifera* L. cv. Superior Seedless, tratadas con IBA 20 ppm y GA_3 20 ppm en plena floración y control.

Table 3 (page 171) shows that the post-harvest quality was improved by IBA and GA_3 treatments. Rachis browning was reduced by applications of IBA consistently during both growing seasons. During the first season, browning was reduced by IBA at full bloom (all doses) and at berry pea size (20 ppm), while in the second season it was reduced by all IBA treatments at full bloom, fruit set and berry pea size. In addition, these treatments showed the highest values of rachis thickness at harvest (table 2, page 169).

Berry shatter was less than 1% during both seasons in bunches treated with IBA (all doses). On the other hand, GA_3 (20 and 50 ppm) at full bloom increased shatter in more than 1%, in correlation with thicker pedicels (table 2, page 169).

Post-harvest berry firmness was increased by both PGRs, as compared to control and in correspondence with berry size at harvest (table 1, page 168).

Bunch fresh weight (FW), berry FW, number of berries per bunch (#Berries), berry diameter; and the xylem and phloem areas, in the different IBA and GA_3 treatments and controls, season 2012/2013.

Peso fresco (FW) del racimo, FW de la baya, número de bayas por racimo (#Berries), diámetro de la baya; y áreas de xilema y floema en los diferentes tratamientos IBA, GA_3 y control, temporada 2012/2013.

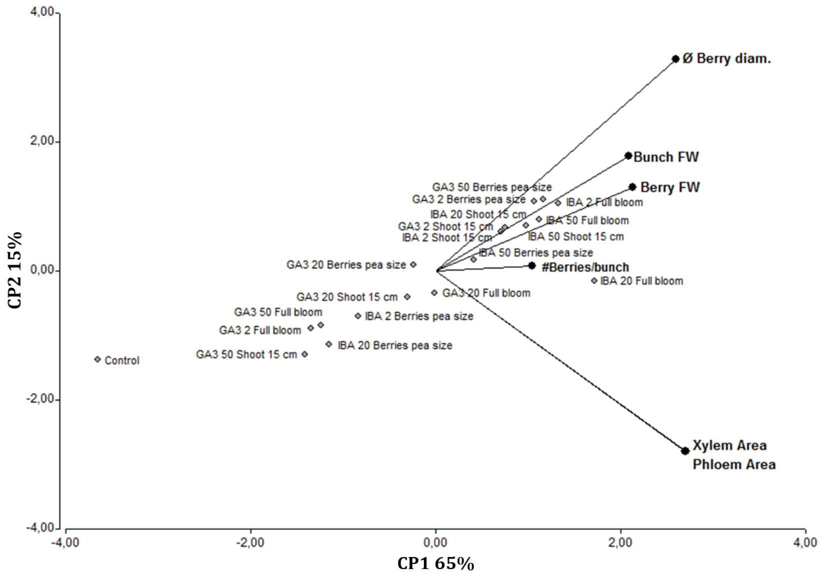

Figure 3. Biplot display of principal component analysis (PCA) of the yield components.

Figura 3. Biplot del análisis de componentes principales (PCA) de componentes del rendimiento.

Table 3. Bunch quality at postharvest: rachis browning, berry shatter and berry firmness of *Vitis vinifera* L. cv. Superior Seedless plants treated with GA_3 and IBA *vs.* control, season 2012/2013 and 2014/2015. PGRs were applied at different phenological stages and doses.

Tabla 3. Calidad de racimos en poscosecha: pardeamiento de raquis, caída y firmeza de bayas en plantas tratadas con IBA y GA_3 en comparación con el control en *Vitis vinifera* L. cv. Superior Seedless, temporada 2012/2013 y 2014/2015.

PGR	Stage	Dose (ppm)	2012/2013 Season			2014/2015 Season		
			Rachis browning	Berry shattering (%)	Berry firmness (N)	Rachis browning	Berry shattering (%)	Berry firmness (N)
GA_3	Shoot 15 cm	2	2.87	0.81	16.47*	-	-	-
		20	2.94	0.24	15.78*	-	-	-
		50	2.88	0.00	16.28	-	-	-
	Full bloom	2	2.93	0.58	15.51*	-	-	-
		20	2.94	1.86*	17.35*	-	-	-
		50	3.00	1.87*	17.84*	-	-	-
	Berries pea size	2	2.95	0.52	17.51*	-	-	-
		20	2.85	0.90	16.76*	-	-	-
		50	2.85	0.36	16.27*	-	-	-
IBA	Shoot 15 cm	2	2.79	0.65	15.52*	1.30	0.94*	14.70*
		20	2.89	0.73	16.96*	1.35	0.92*	15.77*
		50	2.91	0.13	16.97*	1.33	0.11	14.01*
	Full bloom	2	2.73*	0.46	17.54*	1.00*	0.75*	15.48*
		20	2.75*	0.63	16.37*	1.15*	0.92*	12.05*
		50	2.71*	0.86	16.47*	1.15*	0.20	13.13*
	Fruit set	2	-	-	-	1.15*	0.23	13.62*
		20	-	-	-	1.15*	0.44	13.52*
		50	-	-	-	1.15*	0.00	12.54*
	Berries pea size	2	2.89	0.55	16.00*	1.15*	0.21	12.05*
		20	2.73*	0.85	16.53*	1.15*	0.24	12.25*
		50	2.88	0.77	17.04*	1.10*	0.37	12.83*
Control			2.91	0.36	13.53	1.60	0.32	11.66
ANOVA P(value)			0.0017	0.00282	0.0057	0.0123	0.0001	0.0002

Applications at different phenological stages and doses were evaluated. Values are means (n=5) and asterisks show significant differences from Control (Fisher´s LSD test; P≤0.05).

Se evaluaron aplicaciones en diferentes estados fenológicos y dosis. Los valores son medias (n=5) y los asteriscos indican diferencias significativas en comparación con el control (Test LSD de Fisher; P≤0,05).

Discussion

The yield components, bunch architecture and compactness of *Vitis vinifera* cv. Superior seedless were modified by IBA treatments. An increased number of berries per bunch achieved by IBA applied at full bloom and fruit set, is in correspondence with previous findings where IAA increased fruit set. In fact, Costantini *et al.* (2007) found that 'Thompson Seedless' and 'Silcora' (both varieties genetically engineered with an ovule-specific auxin-synthesizing gen (DefH9-iaaM), that increased IAA levels in flowers and fruits) had higher fertility and increased yield, with 30% and 15% increase in berry number, respectively. Tecchio *et al.* (2005) also found that IBA applied at 50 ppm mixed with kinetin (cytokinin) and GA_3 applied 15 days after full bloom on 'Tieta' (Suffolk Red Seedless) increased berry number per bunch. The current results suggest that deficiencies in endogenous auxins associated with embryo abortion at early stages of development in Superior Seedless (stenospermoscarpic fruits) can be corrected by exogenous IBA applications.

Most significantly, bunch FW, berry FW and the number of berries per bunch were increased by IBA treatments at full bloom and fruit set, in association with higher rachis vascular tissue area. In addition, those treatments did not generate bunch compactness, since rachis internode and lateral length also augmented. These effects correlate with the reported role of auxins in promoting cell division, cell expansion (13) and vascular development (21). Guzmán *et al.* (2021) reported an increase in the total vascular bundle area (xylem and phloem) and rachis hydraulic conductivity with IBA plus GA_3 application at full bloom in Superior Seedless. Xylem to phloem ratios are affected by auxin concentration. High auxin concentrations induce xylem as well as phloem, but at low auxin concentrations, only phloem differentiation occurs (1), possibly explaining the reduced xylem to phloem ratio of our control treatment. Furthermore, Else *et al.* (2004) reported an increase in the production of vascular tissues in rachis and pedicels dependent of polar auxin transport through pedicels in wild cherry (*Prunus avium* L.) allowing the fruit to get more water and nutrients. This could explain how, even though the number of berries was increased, TSS were not affected by IBA treatment. During the first season, GA_3 50 ppm at full bloom showed higher TSS than any other treatment. This result may depend on the smaller number of berries per bunch in this treatment, (33% and 46% fewer berries than the control and IBA treatments at full bloom, respectively).

Seasonal effects on bunch FW and berry FW were probably given by weather conditions during bunch and berries growth period. During 2014, daily mean air temperatures were higher than in 2012 (31 °C *vs* 28 °C), surpassing 35 °C (maximum temperature) throughout November and December. These temperatures exceed the photosynthetic optimum for grapevine (below 30 °C), affecting growth conditions. In addition, higher night-time mean air temperatures were also registered in 2014 (28 °C vs 25 °C in 2012), possibly increasing the proportion of respired assimilates (19).

Postharvest quality was augmented by IBA, since rachis fresh weight and diameter were improved in correlation with augmented vascular tissues, and reduced rachis browning. The results coincide with those obtained by Mulet *et al.* (2017) for Superior Seedless with application of IAA at 8-10 mm berry size (berry pea size). However, the treatment IBA at 15 cm shoot length increased rachis diameter but did not have any effect on rachis browning. A similar effect was obtained by Raban *et al.* (2013) with applications of GA_3 and CPPU (cytokinin) in Superior Seedless, Redglobe and Crimson Seedless, where rachis thickness was increased with no browning reductions. Nevertheless, since our results were not consistent, further research is needed to clarify this finding.

Berry shatter was increased by GA_3 at full bloom, in correlation with thicker pedicels. The results suggest that pedicel thickness enhances shatter, possibly given by a lesser pedicel flexibility (34). García-Rojas *et al.* (2018) found that GA_3 applications produced an over-expression of key genes for pedicel lignification and an increase in berry drop. However, considering the fact that up to 3% of shattering is currently accepted for high quality table grape commercialization, none of the PGRs treatments represent an inconvenience since maximum shattering were under 1%(35).

Both PGRs improved berry firmness. Marzouk and Kassem (2011) reported an increase in berry firmness after application of gibberellins in the early stages of fruit growth (4-5 mm fruitlet diameter, and veraison) of Thompson Seedless, then related to berry quality.

Guzmán *et al.* (2021) improved berries firmness and freshness appearance after 60 days cold storage with IBA plus GA_3 in Superior Seedless at full bloom.

Conclusions

The results presented here support the use of IBA as an alternative to GA_3, to improve yield components and bunch quality of Superior Seedless. The proposed alternative, spraying IBA at full bloom and at fruit set, enhanced bunch weight by increasing berry size and weight, in correlation with the promotion of vascular tissue area. In addition, IBA treatments increased the number of berries per bunch, without generating compactness. These results, may be as well beneficial for cultivars that naturally are prone to poor fruit set. Moreover, IBA generates positive effect on postharvest quality via reduction of rachis browning and increase of berry firmness. Finally, it is to be considered that IBA constitutes a plant growth regulator, synthesized by plants, less expensive and while lacking GA_3 negative effects. It will be interesting to find out in future studies, the specific IBA doses and phenological stages application in different table grapes varieties.

Supplementary material

https://drive.google.com/file/d/1w3j3_zR_BNR1Fp9OVUeOjVqWmgt1qBOd/view?usp=sharing

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Authors Contribution

MBP, RB and FB developed the original project, experiment design and prepared the draft manuscript. MBP, YG and DP conducted the experiment in the field and evaluated the yield and bunch architecture. JHA and EA collaborated in development of the experiment design and protocol for assessing bunch structure. YG and CT carried out the anatomical analysis. MBP and FB analyzed the data. All authors reviewed, edited and approved the final version of the manuscript.

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Review

How can nutritional additives modify ruminant nutrition?

¿Cómo pueden los aditivos nutricionales modificar la nutrición de los rumiantes?

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Abstract

This review illustrates the relationships between additives in the diets of ruminants and the consequence for ruminant nutrition. Feed additives are used to improve animal performance and/or the quality of the products. There are several categories of additives available for ruminant nutrition, with emphasis on antibiotics, prebiotics, probiotics, plant extracts, and exogenous enzymes. Antibiotics, essential oils, and chitosan act by selecting sensitive bacteria, consequently modulating the ruminal fermentation pattern. Prebiotics favor microbial growth, providing greater digestion and the production of volatile fatty acids. Probiotics are living microorganisms that improve the ruminal environment, promoting microbial growth and resulting in increased digestion and the production of volatile fatty acids. Exogenous enzymes act synergistically with the enzymes secreted by the ruminal microorganisms, besides favoring microbial adhesion and colonization and facilitating feed degradation. Tannins, whether altering the fermentation standard and/or modifying the ruminal microbiota population, are effective in improving animal performance. However, the effects of additives on the quality of the products are linked to diet quality.

Keywords
chitosan • essential oils • exogenous enzymes • ionophores • yeasts • tannin

Resumen

Esta revisión tuvo como objetivo exponer las relaciones entre los aditivos en la dieta de los rumiantes y las consecuencias causadas en su nutrición. Los aditivos alimentarios se utilizan en la nutrición de los rumiantes para mejorar las características productivas y/o la calidad de los productos. Hay diversas categorías de aditivos disponibles para la nutrición de rumiantes, con énfasis en antibióticos, prebióticos, probióticos, extractos de plantas y enzimas exógenas. Los antibióticos, los aceites esenciales y el quitosano actúan seleccionando bacterias sensibles y consecuentemente modulan el patrón de fermentación ruminal. Los prebióticos favorecen la microbiota, proporcionando una mayor digestión y producción de ácidos grasos volátiles. Los probióticos son microorganismos que mejoran el ambiente ruminal, lo que resulta en una mayor digestión y producción de ácidos grasos volátiles. Las enzimas exógenas actúan sinérgicamente con las enzimas secretadas por los microorganismos ruminales, además de favorecer la adhesión microbiana y colonización de las partículas, ayudan en la degradación del alimento. Los taninos, ya sea alterando el estándar de fermentación y/o modificando la microbiota ruminal, todos los aditivos son efectivos para mejorar el rendimiento animal. Sin embargo, los efectos de los aditivos sobre los productos están relacionados con la calidad de la dieta.

Palabras clave
quitosano • aceites esenciales • enzimas exógenas • ionóforos • levaduras • tanino

Introduction

Ruminants live in symbiosis with the microorganisms that inhabit their rumen. Therefore, the nutrition of ruminants is directly related to microbial fermentation, which occurs in the reticulum-rumen (66). However, the population demographics of these microorganisms promote differences in fermentation, and therefore, the manipulation of this microbial community can be carried out in several ways (37). One is via feed additives, which can facilitate the digestion of the diet components (66). Nutritional manipulation modulating the rumen environment has direct effects on the characteristics of ruminant products (26).

Additives are, by definition, substances, microorganisms, or formulated products added intentionally, which may or may not have nutritional value in the diet (43). Additives used in ruminant diets can be categorized as antibiotics, *i.e.,* monensin, lasalocide, and salinomycin; prebiotics, *i.e.*, plant extracts, tannins, exogenous enzymes, and essential oils; probiotics, *i.e.*, yeasts (30).

Given the above, this review describes the main additives used in ruminant nutrition and discusses their effects on animal performance and animal product quality.

Factors influencing postpartum anoestrus

Ionophores

Ionophores have originally been used as coccidiostats in poultry, but later, it was observed that cattle fed chicken litter contaminated with ionophore residues showed improvement in feed efficiency due to changes in rumen fermentation (9). Ionophores are highly lipophilic polyesters with a molecular weight between 500 and 2,000 Daltons; they can accumulate in cell membranes and modify ionic transport (55) as they are able to act as transporters of different ions and protons across the phospholipid membrane of gram-positive and protozoan bacteria (figure 1).

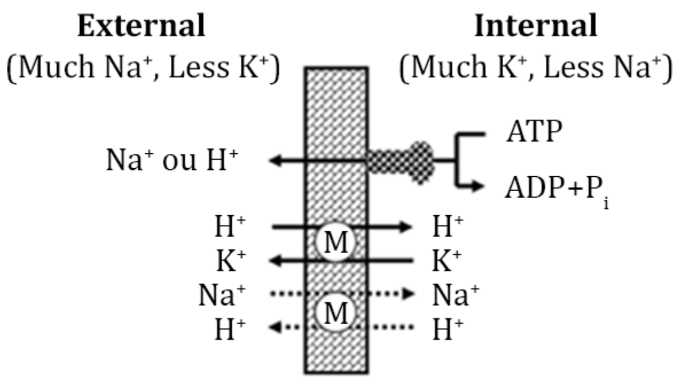


Figure 1. Mechanism of action of ionophores (M- monensin) on the cell membrane (adapted from Russell & Strobel, 1989).

Figura 1. Mecanismo de acción de los ionóforos (M- monensin) sobre la membrana celular (adaptado de Russell & Strobel, 1989).

Due to the need of microorganisms to maintain their membrane gradient, protons must be Na^+ ions, which flow into the intracellular medium and must be expelled through ATPase. However, this action is energetically costly, which raises the energy need to maintain microorganisms, compromising cell proliferation and causing bacterial death (55). In addition, the higher concentration of cations within the bacterial cell increases osmotic pressure and attracts excess water, which results in cell swelling and, consequently, bacterial lysis. The action of ionophores is generally more effective at a pH lower than the pKa value of the ionophores (monensin = 7.95), which occurs because the rumen pH favors the penetration of the bacterial cell membrane ionophore (9). Gram-positive bacteria and protozoa are more sensitive to ionophores because they do not have an outer membrane that provides protection, in contrast to gram-negative bacteria (9, 55).

The use of ionophores as a feed additive is common in ruminant nutrition, especially in dairy and beef cattle. Their inclusion in diets generally causes a reduction in dry matter intake and milk production but an increased body weight gain (1). Such improvements are associated with improved energy and protein metabolism, in addition to an improved health status. The effect of ionophores on energy metabolism is closely related to their ability to facilitate the growth of gram-negative rather than gram-positive bacteria and protozoa, resulting in a lower acetate: propionate ratio, with a consequent increase in the flow of gluconeogenic precursors (2) and a lower availability of H_2 ions, resulting in less energy loss via methanogenesis (1, 36).

The effects of ionophores on energy metabolites have been investigated by Duffield *et al.* (2008) through a meta-analytical study, in which monensin supplementation reduced the serum concentrations of non-esterified fatty acids, beta-hydroxide butyrate acid, and acetoacetate in dairy cows, suggesting less mobilization of body reserves in relation to diets without monensin supplementation. The same authors stated that the supply of gluconeogenic precursors favors the improvement of the animals' energy status, reducing fat mobilization.

Appuhamy *et al*. (2013) performed a meta-analysis of 123 papers, covering the period 1970 to 2011, and observed that monensin supplementation reduced methane production by 7 g/day for dairy cows and 19 g/day for steers, representing a reduction in energy losses.

Other authors also reported that ionophore supplementation reduces methane production (36). In addition to the effects on methanogenesis, Appuhamy *et al*. (2013) also observed that supplemented animals had a lower dry matter intake and an increased milk production, indicating a higher efficiency than in non-supplemented animals. Similarly, related by Golder & Lean (2016) in meta-analytical study. Thus, in cases with beef cattle, monensin supplementation reduced dry matter intake, promoted greater weight gain, and improved feed efficiency (1, 6, 32, 36). Duffield *et al*. (2008) observed that ionophore supplementation decreased the protein and fat percentages of milk; however, fat yield did not change, whereas protein yield was increased with ionophore supplementation. The reduction in milk fat percentage could be related to fat dilution due to an increase in milk production promoted by ionophore inclusion in the diet.

Despite the aforementioned effects on milk composition, the responses appear to be heterogeneous and may be related to the interaction of ionophores with the composition of the diet. A recent meta-analysis (25) has shown that even with greater milk production, protein and fat percentages, as well as protein and fat yield, are not affected by ionophore supplementation. The same authors also did not observe any effects on carcass fat percentage, subcutaneous fat thickness, and marbling degree in beef cattle. Lemos *et al*. (2016) supplemented finishing cattle with ionophores in high-concentrate diets and observed no effects on carcass characteristics.

The fatty acid profile of products from ruminants supplemented with ionophores can be changed by the ionophore effect on the bacterial population, with a consequent effect on the biohydrogenation of unsaturated fatty acids in the rumen. Changes in the fatty acid profile were observed by Duffield *et al*. (2008), show reported a reduction in saturated fatty acids and an increase in unsaturated fatty acids, particularly in the conjugated linoleic acid concentration of animals fed with diets containing ionophores. Despite the beneficial effects of ionophores on the fatty acid profile, such benefits are partly dependent on an adequate substrate supply (polyunsaturated fatty acids) for biohydrogenation (50).

Although ionophores bring benefits to animal production, in 2006, the European Union has banned the use of these products due to public concerns about the use of antibiotics, fearing that the bacteria would acquire resistance and compromise the health of animal product consumers when animals were supplemented with ionophores. In this sense, several other additives have been produced and tested in the feeding of ruminants, particularly yeasts, enzymatic complexes, essential oils, plant extracts, and chitosan.

Yeasts

Against the background of the growing demand for animal products from organic production, studies have been developed to improve production and efficiency in these systems (45), highlighting the use of yeast (*i.e., Saccharomyces cerevisiae*).

There are two main categories of yeasts commercially available for use in animal feed, classified based on the active ingredients and their modes of action. The first category represents yeast cultures; they are not dependent on live yeasts to exert physiological effects after ingestion and provide a mixture of micronutrients composed of soluble cell contents, vitamins, minerals, proteins, peptides, amino acids, lipids, organic acids, esters and alcohols, B vitamins, polyphenols, organic acids, antioxidants, and yeast cell wall compounds such as β-glucans and mannan oligosaccharides. Although these micronutrients can stimulate bacterial growth in the rumen, increase fermentative capacity, and favor animal performance; however, the effects of supplementation with yeast products have mainly been attributed to β-glucans and mannan oligosaccharides present in the yeast cell wall (4).

The second category is represented by products with live yeast cells (more than 15 billion cells/g of product), and the improvement observed with the supply of live yeasts is associated with the removal of oxygen from the rumen environment since yeasts can perform aerobic respiration. Similar to fermentation, the necessary energy for microbial growth is generated, and the intake of traces of oxygen favors the growth of strictly anaerobic bacteria, such as fiber-degrading bacteria (5). However, it is valid to point out that despite the benefits promoted by live yeasts on rumen environment, the life span of these microorganisms in the rumen is approximately 30 hours. If the yeast population is not renewed, the concentrations of these microorganisms in the rumen are reduced to undetectable levels after 4 to 5 days (5) since the optimal pH for yeast growth is 4.5 and the ruminal pH is approximately neutral (7.0), requiring constant yeast supplementation. Based on the short lifespan of yeasts in the rumen, the effects of live yeast supplementation are similar to those of yeast culture supplementation (supply of micronutrients), indicating that the effects of both types of yeasts are similar.

The effects of yeast supplementation on several species of ruminants (cattle, buffalo, goats, and sheep) were evaluated by Desnoyers *et al*. (2009) in a meta-analysis including more than 157 experiments. These authors observed a greater intake and digestibility of dry matter as well as a higher concentration of volatile fatty acids and a higher pH. Similar results were observed by Poppy *et al*. (2012) in a meta-analysis conducted to evaluate the effects of yeast supplementation on lactating dairy cows. The authors found an increase in dry matter intake in early lactation, which is of great importance because in this period, the cows are in an energy deficit, which generally results in a decreased DMI. The authors also observed a reduction in dry matter intake in the final third of lactation, but in both cases, supplementation with yeast increased milk, fat and protein yields, expressed in kg/day, indicating an increased feed use efficiency.

Yeast does not directly affect rumen pH; it impacts the ruminal microflora by stimulating the growth of bacteria that use lactate, *i.e.*, *Megasphaera elsdenii, Selenomonas ruminantium* (13) and *Anaerovibrio lipolytica* (5). The use of lactate as a carbon source by bacteria reduces its concentration in the rumen environment, resulting in a lower pH. This modification favors the growth of cellulolytic bacteria, causing greater fiber digestion and the production of volatile fatty acids. In addition to pH stabilization, the use of lactate as an energy source will give rise to propionate, as the final product of lactate fermentation (42), increasing the energy supply to the host animal (figure 2).

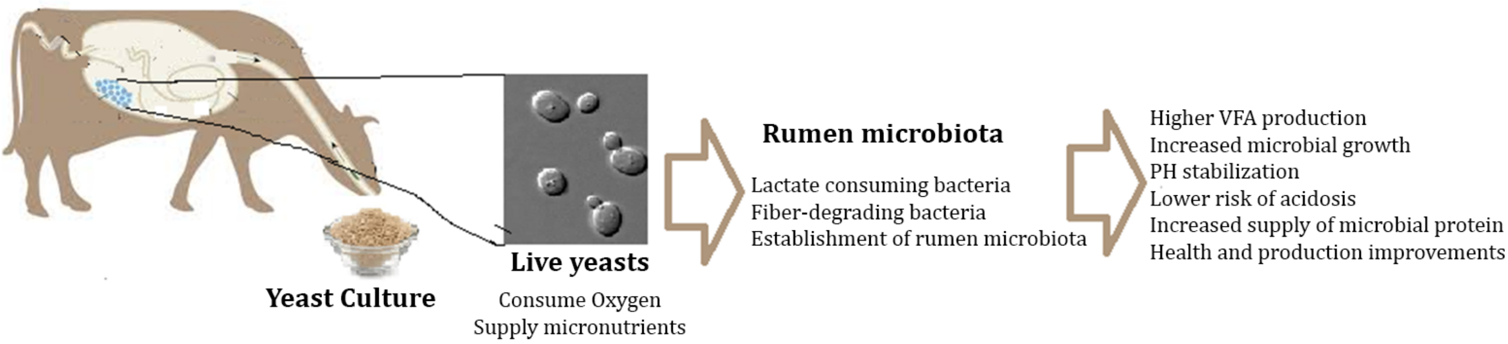


Figure 2. Scheme explaining the modes of action of yeast on rumen microbiota and the consequences for rumen fermentation (adapted from Chaucheyras-Durand *et al*., 2008).

Figura 2. Esquema propuesto para explicar los modos de acción de la levadura sobre la microbiota ruminal y sus consecuencias sobre la fermentación ruminal (Adaptado de Chaucheyras-Durand *et al*., 2008).

Increased cellulolytic population, fiber digestion, and the increased production of volatile fatty acids are also associated with reduced residual oxygen in the rumen environment and/or with the supply of micronutrients. This has been observed by Zhu *et al*. (2017) when they supplied yeast products and verified an increased growth of the cellulolytic bacterial population, mainly *Ruminococus flavefaciens* and *Fibrobacter succinogenes*. However, in this research, there were no effects of supplementation on pH, indicating that the population increase is related to the supply of micronutrients present in yeast cultures. Another important point to be considered is that the increase in the growth of bacteria in the rumen increases the flow of microbial nitrogen, implying a greater supply of amino acids and facilitating animal performance.

The improvements observed by Desnoyers *et al*. (2009), Poppy *et al*. (2012), and Rossow *et al*. (2018) in terms of productive characteristics were not observed in several other studies (33); these discrepancies indicate that the effects of yeast supplementation are dependent on several factors, such as the yeast strain used (54), the viability in the case of live yeasts, diet composition, and the physiological status. Overall, the mechanisms that explain the link between yeast supplementation and increased productivity are still unclear.

Fibrolytic enzymes

Enzymes are proteins acting as biological catalysts and are involved in all biological reactions, accelerating, under specific conditions of humidity, temperature, and pH, the chemical reactions in the cells of living organisms (41). Ruminants fed with roughage are dependent on the synthesis and secretion of enzymes by bacteria, fungi, and protozoa, enabling and accelerating the fermentation of fibrous compounds present in the diet through the action of cellulases, xylanases, β-glucanases (58).

The exogenous fibrolytic enzymes used in ruminants, mainly cellulases and xylanases, refer to a class of enzymes produced by fungal (mainly *Trichoderma longibrachiatum*, *Aspergillus niger,* and *A. oryzae*) and bacterial sources (*Bacillus spp., Penicillium funiculosum*) with high cellulosic and hemicellulosic activity, which can be incorporated in liquid or granular form in the total mixed ration or separately added on roughage and concentrated feeds, as well as on supplements and mineral vitamin premix (3).

Cellulase is the most prominent group of hydrolytic enzymes and catalyzes the hydrolysis of β-1,4 bonds in cellulose. Cellulose degradation is complex and involves several specific enzymes that contribute to cellulase activity (3). In general, endocellulases (endoglucanase, endo-β-1,4-glucanase, carboxymethyl cellulase or β-1,4-glucan, and glucan hydrolase) randomly cleave internal glycosidic bonds in the cellulose chain to produce cellulose oligomers of various degrees of polymerization, whereas exocellulases (exoglucanase, exo-β-1,4-glucanase, β-1,4-cellobiosidase, cellulose) hydrolyze the cellulose chain non-reducing end, yielding cellobiose, and β-glucosidases (cellobiase or glucohydrolase) (38) hydrolyze oligomers of short-chain cellulose and cellobiose to glucose (figure 3).

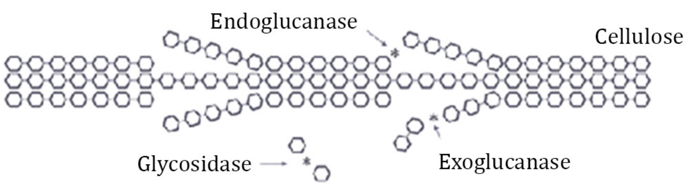


Figure 3. Schematic representation of cellulose degradation to glucose by the sequential action of endoglucanases, exoglucanase, and glycosidases. Asterisks indicate the points of enzymatic cleavage/action (Adapted from Kozloski, 2017).

Figura 3. Representación esquemática de la degradación de la celulosa a glucosa por la acción secuencial de endoglucanasas, exoglucanasa y glicosidasas. Los asteriscos indican el punto de escisión / acción enzimática (Adaptado de Kozloski, 2017).

The effects of fibrolytic enzyme supplementation have been observed by several authors (49). For example, Arriola *et al*. (2011) observed that dairy cows fed low-concentrate diets supplemented with fibrolytic enzymes used the feed more efficiently than cows fed high-concentrate diets without enzyme supplementation due to an increase in ruminal pH, which reduced the risk of acidosis by increasing ruminal pH without altering the production of volatile fatty acids and milk. These authors reported an increase in feed efficiency due to a lower dry matter intake with enzyme supplementation. Lunagariya *et al*. (2020) reported that dry matter and nutrient intake was not influenced by enzyme supplementation. However, the authors observed an increase in feed efficiency, the digestibility of nutritional fractions, and body weight gain in non-pregnant Gir and crossbred dairy cows.

Supplementation with fibrolytic enzymes also improved the average daily gain, the use of feed nutrients and feed efficiency, explained by the greater digestion of fibrous components, and resulted in an improved digestion of the fibrous fraction and in the production of volatile fatty acids (22). Chung *et al*. (2012) reported that enzyme supplementation increased the number of fibrolytic bacteria (*Fibrobacter succinogenes*) compared to that of non-fibrolytic ones (*Ruminobacter amylophilus*).

The increase in feed digestion is probably not simply the result of supplemental enzyme activity, whereas the contribution of exogenous enzymes added to diets to the overall rumen activity is relatively small (30). Probably, the exogenous enzymes act synergistically with the enzymes secreted by ruminal microorganisms, resulting in a more pronounced fibrolytic effect in the rumen environment (58). Concomitantly, improved digestibility is associated with the breaking of physical fiber barriers during the digestion process, favoring microbial access and colonization (58), which result in a higher number of microorganisms in the diets added with enzymes (16).

However, it is important to note that while the supply of exogenous fibrolytic enzymes may increase the degradation rate and the extent of fiber degradation in the rumen (51), the absence of positive results may be related to the animals' physiological state, the supply mode, the supply amount, and the characteristics of the used enzymes (3, 11). According to Adesogan *et al*. (2014), the exogenous fibrolytic enzyme considered ideal for improving performance must have the following characteristics: a) contain appropriate substances to increase fibrolytic activity and improve fiber digestibility; b) contain appropriate amounts of cofactors, coenzymes, and activators (when necessary) to optimize the activities of fibrolytic enzymes without inhibiting them; c) not be susceptible to degradation by ruminal microorganisms or hydrolysis by plant, ruminal, or microbial proteases; d) present a robust composition so that it does not show significant variation from one batch to another; e) be produced from fungi or bacteria that produce large amounts of enzymes, either naturally or through genetic modification; f) present optimum and stable activity under the conditions of the place where it will exert its hydrolytic effect; g) must be in liquid form or dissolve quickly and completely in water; h) be thermostable if added during the manufacture of feed; i) maintain its hydrolytic activity when properly stored for long periods; j) be considered safe for use. In addition Adesogan *et al*. (2014) also pointed out that additional factors could explain the variability of the results of experiments with exogenous fibrolytic enzymes, such as inadequate designs, low statistical power, and short-term experiments.

Arriola *et al*. (2017), in a meta-analytical study evaluating effects of supplementation with fibrolytic enzymes in diets for dairy cows, observed that the supply of exogenous enzymes did not alter the daily dry matter intake and feed efficiency but tended to increase dry matter and neutral detergent fiber digestibility. The improvement in digestibility promoted discrete increments in milk production, the production of 3.5% fat-corrected milk (kg/day) of protein, lactose, and fat; in contrast, supplementation reduced the percentage of fat milk, which is justified by the dilutive effect promoted by the greater milk production (7). In another meta-analytical study, Tirado-González *et al*. (2018) observed results similar to those of Arriola *et al*. (2017), in which enzyme supplementation provided greater milk production as well as protein and fat yields, in addition to a higher average daily gain in beef cattle.

Essential oils

In recent years, there has been a considerable interest in the use of essential oils, driven by the concerns caused by the use of ionophores, which may enable increased resistance to antibiotics. Essential oils are natural, complex, and volatile compounds with a strong odor. They are synthesized from different plants by distillation drag with water vapor or solvent extraction (20).

Essential oils can be used as additives in ruminant nutrition, modulating the rumen environment similar to antibiotic additives (28). Figure 4 (page 182) illustrates the mechanism of antimicrobial action of essential oils on the bacterial cell.

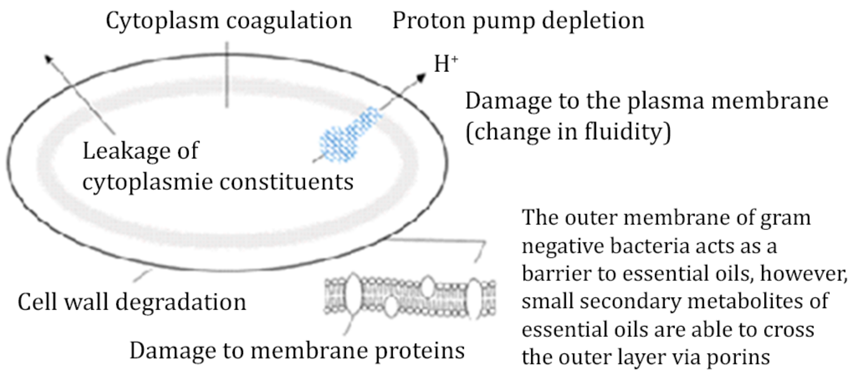


Figure 4. Mechanism of antimicrobial action of essential oils on the bacteria (Adapted from Burt, 2004).

Figura 4. Mecanismo de acción antimicrobiana de los aceites esenciales sobre las bacterias (Adaptado de Burt, 2004).

Essential oils are hydrophobic but lipophilic, departing from the aqueous phase into the lipid bilayer of the cytoplasmic membrane of bacteria and accumulating and interacting with their membranes (12, 17). Consequently, the membrane becomes more permeable, enabling ion translocation and, hence a decrease in the ionic gradient. Furthermore, essential oils can also interact with membrane proteins and/or other cytoplasmic components (10). To track changes in the ionic gradient, the bacteria use the ion pump to drive the ions; however, such action demands high amounts of energy, affecting bacterial growth (10). The interaction of essential oils with the cytoplasmic components can cause cytoplasmic coagulation and lead to cell lysis.

Combinations of different essential oils modify their individual effects because of the possible synergism between compounds with different functions and mechanisms of action and the interaction with other active ingredients, amplifying the effect when mixed (10). Some types of essential oils stimulate rumen fermentation, whereas others inhibit methanogenesis, increase production, and change the profiles of volatile fatty acids and nitrogen metabolism (12).

Valero *et al*. (2016) observed increased feed efficiency and average daily gain in cattle supplemented with essential oils, but without changes in carcass characteristics and meat quality. Similar results were observed by Ornaghi *et al*. (2017), who tested two types (cinnamon and clove) and two doses (3.5 and 7.0 g/day) of essential oils in diets for Nellore cattle. These authors attributed the positive effects on the antimicrobial properties and the presence of volatile and odorous compounds from the essential oils, with a greater dry matter intake, showing an improved acceptability of the diet. An increased acceptability of diets with essential oils has also been reported by other authors (20).

Improvements in performance and feed efficiency were observed by Moura *et al*. (2017), who included 0.5 g/kg of dry matter in finishing lamb diets. According to the authors, copaiba essential oil had a greater antimicrobial activity toward gram-positive bacteria than gram-negative ones, leading to changes in the microbial population. Ornaghi *et al*. (2017) stated that as gram-negative bacteria are more likely to produce propionate, a gluconeogenic precursor, the availability of glucose is increased, which may justify the greater weight gain. However, such effects of essential oils on rumen microbial populations are still inconclusive (57).

The effects of essential oils were also tested by Tomkins *et al*. (2015), who used the commercial product CRINA®, composed of a mixture of thymol, eugenol, vanillin, limonene, and guaiacol; the authors observed no effects on the microbial population, the production of volatile fatty acids, and methane production. According to the authors, the essential oils can be removed during normal rumen flow rates, and the dosage provided can be reduced to minimum concentrations that do not inhibit microorganisms. In addition, since essential oils have a low solubility, they can accumulate in the rumen environment and thus have a low effect.

Another possible explanation for the various results found in the literature is the adaptive capacity of rumen microorganisms to essential oils when supplied at low concentrations (10). The absence of the effect of essential oil supplementation was also verified by Simitzis *et al*. (2014), who evaluated the inclusion of oils in the diets of lambs and did not observe effects on feed intake, performance, and meat quality.

Several factors may affect the responses of supplementation with essential oils, namely the plant species used for the extraction of essential oils, the growth phase of the plant, the plant part (leaves, bark, flowers, roots, seeds), the growth environment, as well as soil composition, temperature, and light and water stress (10, 12).

Moreno *et al.* (2021) reported that the inclusion of *Allium sativa* when associated in diets due to its organosulphurous compounds as allicin, which have antimicrobial activities act modulating the function of the rumen environment and favoring the synthesis of sulfur amino acids as methionine by the contribution of these compounds.

Rivaroli *et al*. (2016) evaluated the quality of the meat from animals consuming essential oils and observed that low doses reduce lipid oxidation and increase the shelf life of meat, when compared to diets without essential oils, due to the antioxidant effects of essential oils. In contrast, in high doses, essential oils act as pro-oxidants as they can increase the permeability of mitochondria, thereby damaging them and altering electron flow, which results in the production of more free radicals, such as reactive oxygen species (10).

Despite the demonstrated effects on the microbial population and, consequently, fermentation and animal performance, these effects have only been studied for a limited number of oils, necessitating more studies evaluating the potential of different types of essential oils in animal feed. However, secondary compounds from plants, *i.e.*, essential oils and tannins, can be used as natural products, without the issues mentioned for ionophores in ruminant production.

Tannins

Tannins are secondary compounds of plants characterized by phenolic rings and of variable molecular weight. They can form complexes with various substances due to the different forms of interactions such as covalent bonds, hydrogen bonds, or ionic bonds (61). These compounds are stored in the vacuoles of plant cells and have a defense function because of their antimicrobial and/or bacteriostatic characteristics, helping the plant reabsorb nutrients from injured cells or by inhibiting the action of predators (61).

Because of the antimicrobial and/or bacteriostatic properties, the use of tannin in the form of extracts or supplied through plants or plant parts containing tannin is widely used as an alternative method of rumen modulation, with the beneficial effects of methane mitigation, changing the biohydrogenation of polyunsaturated fatty acids (47, 50, 61).

Several studies have reported divergences regarding the tolerance of ruminants to the intake of diets containing tannin. In general, the tannin content varies between 4% and 5% of the dry matter of the diet. Supply occurs through plants containing tannin; if tannins are supplied via extracts, they are mainly in liquid or powder form. The chemical characteristics of the tannin and the protein, which are the salivary protein, or the protein from the other dietary components, may largely interact (50).

The use of tannin in ruminant diets *a priori* emerged as a way to increase the efficiency of the use of dietary nitrogen, mainly because through the tannin-protein bond, part of the protein is no longer degraded in the rumen and continues to the abomasum, where the reduction in pH promotes the tannin shutdown with the protein, leaving it available to be digested in the jejunum. In this way, the decreased degradation of proteins in the rumen can decrease the concentration of nitrogen in the rumen, in the form of ammonia and/or urea, thus reducing the excretion of urea and promoting nitrogen recycling and the use of essential amino acids in the diet (61). However, the presence of tannin in feed particles has negative impacts on gram-positive bacteria and Archaea, causing changes in the biohydrogenation process (26, 61).

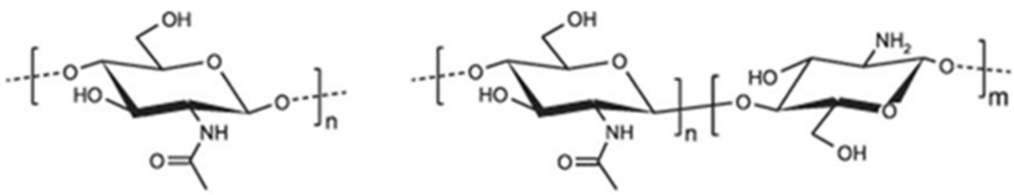
Despite the positive effects mentioned above, some authors have reported that the addition of tannin caused a reduction in dry matter intake and in the digestibility of dry matter, protein, and neutral detergent fiber (27). Villalba *et al*. (2010) reported positive effects of the inclusion of tannins in ruminant diets and explained that because of the antiparasitic action of tannin, there was a greater integrity of the intestinal wall area, favoring the absorption of nutrients and, consequently, increasing the production of meat, milk, and wool. Tannin levels affect the use of the diet (intake and digestibility) and, consequently, the production of meat, milk, and wool. Regarding meat production, Vasta *et al*. (2009) reported an increase in the ruminal fluid of the concentration of conjugated linoleic acid and, consequently, an increase in the concentrations of polyunsaturated fatty acids in the muscles of sheep. In their reviews, Ribeiro *et al*. (2011) and Morales & Ungerfeld (2015) stated that tannin more significantly affects the microorganisms that exert the last step of biohydrogenation, thus increasing the supply of 18: 1 fatty acid isomers to the small intestine.

Patra (2014) reported several studies in which the inclusion of tannin in the diets had no effects on the fatty acid profile of the milk of goats and sheep. However, Morales and Ungerfeld (2015) reported that the inclusion of tannin reduced the concentration of 18: 1 fatty acids and increased those of 18: 2 and 18: 3 fatty acids. In meat, tannin, when included in the lamb diet, increased the deposition of 18: 3, 18: 2, and 18: 1 and reduced the concentrations of saturated fatty acids (39, 47).

When the tannin inclusion is associated with diets containing high levels of polyunsaturated fatty acids, changes in the fatty acid profile of the product are promoted.

Chitosan

Chitin is the second most abundant organic compound on Earth and can be found in the cell walls of some fungi and algae as well as in the exoskeletons of some invertebrates such as crab and shrimp (31). Chitosan (polymer N-acetyl-D-glucosamine) is a natural biopolymer formed by the deacetylation of chitin in an alkaline medium (31) and mainly comprises 2-amino-2-deoxy-D-glycopyranose units. Chitin is composed of 2-acetamido-2-deoxy-D-glycopyranose units, both of which are linked by glycosidic bonds (figure 5) (56).



Chitin Chitosan

Figure 5. Chemical structures of chitin and chitosan (Adapted from Silva *et al*., 2006).

Figura 5. Estructura química de la quitina y el quitosano (adaptado de Silva *et al*., 2006).

Chitosan employment has increased exponentially in several areas, such as agriculture (53) or the food and pharmaceutical industry (11, 15). However, its use as a feed additive in ruminant nutrition is largely neglected (15, 18).

The effect of chitosan as an additive in ruminant nutrition was investigated by Goiri *et al*. (2010), using the rumen simulation technique (RUSITEC). The authors found that diets with chitosan do not alter the total production of volatile fatty acids but facilitate the production of propionate, resulting in a higher propionate: acetate ratio. Such modifications are relevant from an energy perspective and could improve feed efficiency in farm animals. Similar results were reported by Dias *et al*. (2017). An increase in the propionate: acetate ratio was also observed by Vendramini *et al*. (2016), who also found a reduction in methane production and a tendency to reduce fiber digestion, indicating a possible modification of the rumen microbial ecosystem, especially in fibrolytic bacteria.

The effects of chitosan supplementation on ruminal microorganisms were also investigated by Goiri *et al*. (2010), who observed an inhibition of biohydrogenation fatty acids in the rumen, increasing the proportions of $C18{:}1_{t11}$ (vaccenic acid) and conjugated linoleic acid and decreasing the proportions of saturated fatty acids. Alteration of the rumen microbiota can also have negative effects on dry matter digestibility, particularly in diets with a high amount of roughage, indicating that the effects of chitosan are greater on gram-positive bacteria (64).

Paiva *et al*. (2017) evaluated chitosan in the diet of cows and verified an increased milk yield as well as higher lactose and protein yields; however, the authors found no changes in the percentages of milk components. They attributed the high milk production to the greater supply of propionate, a gluconeogenic precursor. Garcia-Rodriguez *et al*. (2015) also reported higher concentrations of plasma glucose, suggesting a greater propionate production. Mingoti *et al*. (2016) reported that chitosan supplementation did not alter intake and milk yield and reported only slight changes in the milk fatty acid profile; these findings are different to those reported by Garcia-Rodriguez *et al*. (2015) and mentioned above.

Gandra *et al*. (2016) reported that chitosan supplementation reduces diet intake but increases dry matter digestion. Mingoti *et al*. (2016) found that diets with chitosan and soybean oil promoted an increase in milk yield and feed efficiency, reducing the profile of saturated fatty acids and increasing the levels of polyunsaturated fatty acids, mainly C18:2 cis-9,12, indicating changes in the biohydrogenation process.

Mingoti *et al*. (2016) explained that chitosan is capable of modifying rumen biohydrogenation, but that the slight change in the profile of milk fatty acids found in this study is more related to the source of polyunsaturated fatty acids (whole soybean), which not being free, did not suffer the direct action of rumen biohydrogenation, because of this change in the profile was discreet considering the minor microbial action in the supply of fatty acids that reached the duodenum.

The mode of action of chitosan on microorganisms is not fully understood, and the theory of the intracellular extravasation mechanism is most accepted by the scientific community. In this theory, the positive charges of chitosan interact with the negative charges on the lipopolysaccharide surface of gram-negative bacteria and, similarly, with the peptidoglycan fraction of gram-positive bacteria (15). These interactions modify membrane permeability and promote the extravasation of intracellular components, causing bacterial death (15, 21). However, these effects are less pronounced in gram-negative bacteria due to the presence of an outer membrane in these microorganisms; such a membrane is absent in gram-positive bacteria (figure 6), making them more susceptible to the action of chitosan.

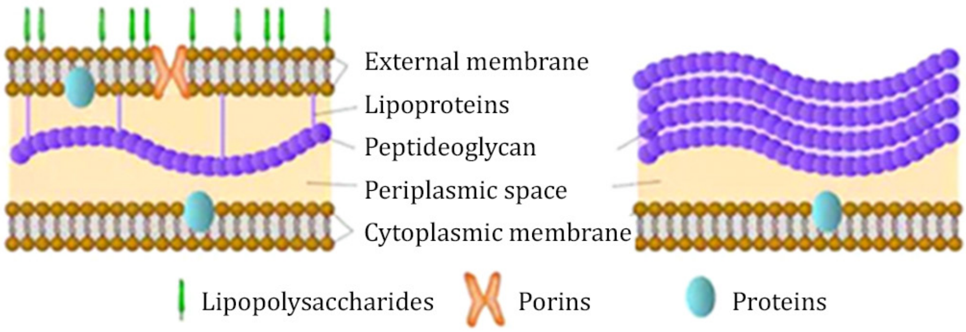


Figure 6. Schematic view of the cell walls of gram-negative (left) and gram-positive bacteria (right) (adapted from Nelson & Cox, 2014).

Figura 6. Vista esquemática de la pared celular de bacterias gramnegativas y grampositivas, respectivamente (Adaptado de Nelson & Cox, 2014).

Conclusion

The additives presented in this review have different mechanisms of action in the rumen environment, either by changing the fermentation pattern and/or modifying the rumen microbiota, all of which are efficient in improving animal performance. There is an intrinsic relationship to the quality of meat and milk from ruminants, and these products are also related to the diet (concentrate: roughage rate, EE content, dietary fatty acid profile, dietary fiber quality).

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