@RevFCAUNCuyo

Rev FCA

Revista de la Facultad de Ciencias Agrarias | UNCuyo

75 años

Tomo 56 Nº 1 | Año 2024
ISSN on line 1853 8665
Mendoza. Argentina

Evaluadores 2024

Ricardo Alcántara de la Cruz
Universidade Federal de Sao Carlos-Brasil

Hudhaifa AL-Hamandi
Tikrit University-Turquía

Juan Aller
INTA

Stella Bogino
UNSan Luis

Boris Camiletti
CONICET

Andrea Karina Cancino
INTA

Walter Ricardo Chale
UNNOBA

Francisco Antonio Cigarroa Vázquez
UAutónoma de Chiapas-México

Juan Cruz Colazo
INTA

Alvar Alonso Cruz Tamayo
UAutónoma de Campeche-México

Hector Daniel Estelrich
UNLa Pampa

Luis Ferré
INTA

Mónica Gaggiotti
INTA

Romina Manfrino
CEPAVE

Carlos Osella
UNLitoral

Roxana Piastrellini
CONICET

Gustavo Polenta
INTA

Cecilia Rébora
UNCuyo

Paulo Recavarren
INTA

Fidelina Silva
INTA

Carla Suárez
UNLa Pampa

María Eugenia Van den Bosch
INTA

María Florencia Vianna
UNLa Plata

Marisa Wawrzkiewicz
UBA

Eduardo Wright
UBA

Marcos Yanniccari
INTA

Revista de la Facultad de Ciencias Agrarias
Universidad Nacional de Cuyo

Tomo 56 (1) - Junio 2024

Índice

Ecofisiología y manejo de cultivo

Recuros naturales y ambiente

Protección vegetal

Selection of *Rhizobium leguminosarum* strains via symbiotic and production variables in *Pisum sativum* L.

Selección de cepas de *Rhizobium leguminosarum* por variables simbióticas y productivas en *Pisum sativum* L.

Carlos Piccinetti [1], Carolina Alba Eöry [2], Gabriel María Prieto [3], Daniela Adriana Vallejo [1], Juan Martín Enrico [4], Fernando Salvagiotti [4], Alejandro Perticari [3]

Originales: *Recepción:* 21/04/2023 - *Aceptación:* 28/11/2023

Abstract

Field pea (*Pisum sativum* L.) is a winter symbiotic legume that associates with *Rhizobium leguminosarum* sv *viciae*. This work aimed to evaluate strains of *R. leguminosarum* for their infective ability and early-plant growth, BNF contribution, biomass and grain yield. Seventy-eight specific strains and four pea cultivars were evaluated in a growth chamber, five strains and three cultivars were evaluated in a greenhouse, and three strains and two cultivars were evaluated in a field experiment. Only 44-55% of all evaluated strains were infective in the four cultivars. In the greenhouse, D70 and D156 strains showed the best nodulation variables as well as higher N content and yield. The field experiment showed D156 and D70 yielded a similar behavior for N content in canopy biomass and individual nodule biomass, whereas D191 had a higher nodule number per plant, aerial biomass and grain yields. D70 provided good nodulation, N content in biomass, and yield in the growth chamber, greenhouse, and field experiments, whereas D156 had a like or superior behavior in the greenhouse and field experiments. Therefore, D156 could constitute a good candidate for bacterial single-strain inoculants, as well as for formulating microbial consortia.

Keywords

Field pea • *Rhizobium leguminosarum* • strain selection • symbiotic efficiency

1 INTA. Instituto Nacional de Tecnología Agropecuaria. Laboratorio Bacterias Promotoras del Crecimiento Vegetal (LBPCV). Instituto de Microbiología y Zoología Agrícola (IMYZA). Nicolás Repetto y De Los Reseros s/n° - Hurlingham (B1686). Buenos Aires. Argentina. * piccinetti.carlos@inta.gob.ar

2 Profesional independiente. Arredondo 3488. Castelar (1712). Buenos Aires. Argentina.

3 Agencia de Extensión Rural Arroyo Seco. Estación Experimental Agropecuaria Oliveros-INTA. San Martin 528. Arroyo Seco (2128). Santa Fe. Argentina.

4 INTA. Manejo de Cultivos, Suelos y Agua. Estación Experimental Agropecuaria Oliveros. Ruta 11 km 353. 2206. Oliveros. Santa Fe. Argentina.

Resumen

La arveja (*Pisum sativum L.*) es una leguminosa simbiótica del invierno que establece asociación con *Rhizobium leguminosarum* sv *viciae*. Los objetivos de trabajo fueron evaluar cepas de *R. leguminosarum* por infectividad y crecimiento temprano, aporte de BNF, producción de biomasa y rendimiento. Setenta y ocho cepas y cuatro cultivares se evaluaron en cámara de crecimiento, cinco cepas y tres cultivares en invernadero, mientras que tres cepas y dos cultivares en experimento de campo. Solo el 44-55% de las cepas fueron infectivas en los cultivares. En invernadero, las cepas D70 y D156 mostraron los mejores valores de nodulación y los mayores contenidos de N en biomasa y rendimiento. En campo, D156 y D70 mostraron comportamientos similares que en invernadero, mientras que D191 tuvo más nódulos por planta, mayor biomasa aérea y mayor rendimiento. La cepa D70 mantuvo su comportamiento de nodulación, contenido de N en biomasa y rendimiento en cámara de crecimiento, invernadero y campo; mientras que D156 tuvo igual o mejor comportamiento que D70 en invernadero y campo. Por lo tanto, D156 podría ser una buena candidata para formular inoculantes bacterianos con esta cepa o para formular consorcios microbianos.

Palabras clave
Arveja • *Rhizobium leguminosarum* • selección de cepas • eficiencia simbiótica

Introduction

Field pea (*Pisum sativum* L.) is a winter symbiotic legume able to establish a specific mutualistic association with *Rhizobium leguminosarum* symbiotic variant (sv) *viciae.* This association contributes to satisfying part of the crop nitrogen (N) demand via biological nitrogen fixation (BNF). From an agronomic perspective, field peas may be included as an alternative winter crop in agricultural rotations, with a shorter cycle duration and lower water consumption than other winter crops like wheat or barley, and the N-fixing ability (19). The N-fixing efficiency of the legume-rhizobium relationship depends mainly on crop genotype (1, 2), rhizobium strain (23), and soil-water interactions associated with management practices (10, 13, 14). Contributions of BNF in field pea range between 45 and 286 kg N ha^{-1} (1, 13, 16, 22, 31, 36), representing 33-91% of total N uptake (1, 13, 16, 21, 22, 26, 31, 36).

Rhizobium leguminosarum strains have three distinct symbionts: (i) *R. leguminosarum* sv *phaseoli*, which nodulates beans (*Phaseolus vulgaris*); (ii) *R. leguminosarum sv trifolii*, which nodulates clovers (*Trifolium* sp); and (iii) *R. leguminosarum sv viciae*, which nodulates field pea, vetch (*Vicia* sp), lentil (*Lens* sp), faba beans (*Vicia faba*) and Lathyrus (*Lathyrus* sp) (7, 8, 27). In field peas, statistical interactions between strains and crop genotypes have been observed for traits associated with BNF, plant growth and photosynthesis (11). Rhizobia strains have greater relevance on BNF and N content, biomass and grains, than plant genotype (23). This strain-dependent behavior is associated with the expression of genes related to *R. leguminosarum* sv *viciae* nodulation, ensuring nodule establishment (7).

In Argentina, although field pea is not a native plant, native soil N-fixing rhizobia can nodulate this crop. Currently, the *R. leguminosarum* native strain D70 is the elite strain inoculating field peas, vetches, and lentils. This strain can nodulate all commercial cultivars and has shown greater BNF contribution in all agro-ecological environments evaluated in Argentina (13). However, even showing good behavior regarding grain yield and biomass production, it does not necessarily respond equally in all agricultural environments. Newly incorporated commercial cultivars still require evaluation regarding symbiotic behavior. In this regard, strains of the collection of the Instituto de Microbiología y Zoología Agrícola (IMyZA) of the Instituto Nacional de Tecnología Agropecuaria (INTA) could potentially perform better than D70 and be used in new inoculant formulations. Thus, this work aimed to i) evaluate *Rhizobium* sp. soil isolates and strains from the IMyZA-INTA collection for infective ability and early growth promotion under controlled conditions (growth chamber), ii) evaluate symbiotic efficiency of strains selected from the growth chamber, under greenhouse conditions, and iii) evaluate inoculation effects of the greenhouse selected strains on BNF contribution, nodulation, and production variables under field conditions.

Materials and methods

Growth chamber experiments

Seventy-one to seventy-eight specific strains and/or soil isolates from the IMyZA-INTA collection (Additonal material 1) were combined with four field pea cultivars (Facón, Bicentenario, Manantiales, and Pampa). *Rhizobium* sp. inocula were grown in glass tubes (20 mm x 195 mm) with 10 ml of yeast extract mannitol (YEM) broth (35) on an orbital shaker (180 rpm, 48 h at 28°C). Each inoculum contained at least 3 x 10^8 colony-forming units per milliliter (CFU ml^{-1}). Field pea seeds were disinfected superficially with 80% ethanol for 1 min, 4% sodium hypochlorite for 2 min, at 80% ethanol for 0.5 min, and rinsed 5 times with sterile distilled water. Then, these seeds were placed in plastic trays with moistened absorbent paper for 72 h in an incubation stove at 28°C for germination. Two germinated seeds were inoculated with 1 ml of inoculant and planted in a 220-ml pot with sterile vermiculite substrate and daily irrigated with N-free nutrient solution (17). Each pot was kept at a constant temperature of 25°C and 16 h of light. Each strain and cultivar combination was considered a treatment, and a non-inoculated control was added for each field pea cultivar. The experimental design was set as completely randomized with three replications. Finally, the number of nodules per plant ($N°_{Nod}$) and biomass per plant ($Biom_{Total}$) were evaluated 25 days after planting (DAP).

Greenhouse experiment

Five strains selected from the growth chamber experiment (D70, D156, D191, D192, and D193) were evaluated for nodulation and early growth promotion abilities in the greenhouse. The experiment was planted in 2 L pots on August 21st, 2013, using four seeds per pot and sterile vermiculite as substrate. Seeds were disinfected as described above. Treatments consisted of a factorial combination of the five selected strains plus a non-inoculated control, with three commercial field pea cultivars (Viper, Facón, and Bicentenario) arranged in a complete randomized block design totaling 17 replicates. The inoculant was peat-based with a count of at least 1 x 10^8 CFU g^{-1} of product. Sugared water (20% w/v) was used as adherent at a rate of 10 ml kg $seed^{-1}$. The inoculant was applied to seeds, mixed to homogenize, and allowed to stand for 30 min before planting. Pots were irrigated three days a week with an N-free nutrient solution (17). Variables per plant were evaluated at 56 DAP and 78, 92, and 103 DAP per pot. Root biomass ($Biom_{Root}$), aerial biomass ($Biom_{Aerial}$), plant biomass ($Biom_{Plant}$), number of nodules ($N°_{Nod}$), dry nodule biomass ($Biom_{Nod}$) and dry biomass per nodule ($Biom_{Nod-I}$) were evaluated at two sampling moments (at 56 and 78 DAP), with additional measurements such as root and aerial length ($Leng_{Root}$ and $Leng_{Stem}$, respectively) at 56 DAP. Then, the following variables were also evaluated: vegetative, grain, and aerial biomass ($Biom_{Veg}$, $Biom_{Grain}$, and $Biom_{Aerial}$, respectively), and plant yield components as pod numbers, number of grains, number of grains per pod, and individual grain biomass ($N°_{Pod}$, $N°_{Grain}$, $N°_{Grain-pod}$, and $Biom_{Grain-I}$, respectively) at harvest (ca. 103 DAP). Additionally, N content ($\%N_{upt}$) and isotopic composition ($\delta^{15}N_{upt}$) on whole plant biomass were determined at 92 DAP to estimate the β factor for each strain and field pea cultivar combination according to Boddey *et al.* (2000).

Field experiment

Strains D70, D156, and D191, first evaluated in the greenhouse, were then compared in a field experiment. All field treatments (*i.e.* the combinations between these three strains and two pea cultivars, Facón and Viper) were carried out in triplicate. The field experiment was performed in the locality of Rueda (33°21'48.48" S and 60°27'39.12" W), in Santa Fe province (Argentina), on a Typic Argiudoll soil. The experimental unit (plot) had six furrows (at 0.175 m interrow) and 5 m in length. The experiment was sown on July 29th, 2014, with 119 plants m^{-2} (110-127 plants m^{-2}) and harvested on November 14th (108 DAP). Plants were fertilized with 100 kg ha^{-1} of monoammonium phosphate (*i.e.* 20 kg P ha^{-1}). Seeds were inoculated as described in the greenhouse experiment. Weeds and pests were controlled. During the cycle, total rainfall was 284 mm and mean temperature was 17.3°C.

At 60 DAP (September 14th), 0.5 m of two rows (0.18 m^{-2}) was sampled and the number of nodules in each plant ($N°_{Nod}$) was counted. Then, plants were oven-dried at 65°C for 72 h to determine nodule biomass per plant. At 95 DAP, 1 m^2 of the total aboveground biomass of field pea of each plot (N-fixing crop) plus one linear meter of wheat (non-fixing crop) were sampled. These samples were dried in an air circulation stove at 65°C for 72 h. A subsample of each mentioned sample was ground with a Wiley mill to determine N content in biomass ($\%N_{upt}$) according to the micro-Kjeldahl method. The N uptake (N_{upt}) was obtained by multiplying $\%N_{upt}$ by $Biom_{Aerial}$. BNF was determined by the ^{15}N natural abundance method, using an elemental analyzer Carlo Erba EA 1108 coupled to a ThermoScientific Delta V Advantage isotopic mass spectrometer of continuous flow through a ConFLo IV interface. Then, $\%N_{FBN}$ was estimated according to Collino *et al.* (2015) and N_{BNF} was calculated by $N_{BNF} = \%N_{BNF} * N_{upt.}$

At harvest (108 DAP), two samples of aboveground biomass ($Biom_{Aerial}$) were taken from each plot of 1 m^2. The, grained ($Biom_{Grain}$) and vegetative structures ($Biom_{Veg}$) were separated, dried in an air circulation stove at 65°C for 72 h and weighed. The harvest index was calculated. Grain yield ($Yield_{Grain}$) was adjusted to grain moisture of 0.135 kg water kg $grain^{-1}$.

Molecular characterization

Three of the strains evaluated (D70, D156 and D191) were characterized by partial sequencing of the 16S rRNA gene by amplification reactions. Pure colonies were grown on plates in YEM culture medium, then suspended in 50 µl of ultrapure water and boiled in a water bath for 10 min to obtain DNA extracts. The universal primers fD1 and rP2 proposed by Weisburg *et al.* (1991) were used and amplification was carried out in a volume of 25 µl of the reaction containing 2.5 ml 1X Buffer, 0.75 µl of 50 mM $MgCl_2$, 0.5 µl of 10 mM dNTP, 0.25 µl Taq DNA Polymerase, 0.5 µl of each primer (fD1 and rP2), 1 µl of tempered genomic DNA and 19 µl of ultrapure water. Amplification conditions consisted of an initial denaturation of 2 min at 94°C, followed by 35 cycles of denaturation (94°C, 40 seconds), annealing (52°C, 40 seconds) and extension (72°C, 1.5 min) and a final extension at 72°C for 10 min. A negative control without template DNA was included. Electrophoresis was performed at 90 V. The amplified products were analyzed by 0.8% agarose electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen™) for 30 min and then purified with the commercial Gel Extraction kit QIAEX II (Qiagen). Partial sequences of 16S rRNA were compared with those deposited in the NCBI BLAST database (http://blast.ncbi.nlm.nih.gov/).

Data analysis

Statistical analysis of the growth chamber, greenhouse, and field experiments was carried out using a two-way ANOVA. Means were compared with the DGC test ($p \leq 0.05$). All analysis was performed using Infostat software version 2018p (Di Rienzo *et al.*, 2018).

Results

Growth chamber experiment

Only 44-55% of all the strains tested (71-78) presented nodules in the four cultivars (*i.e.* they were infective), varying according to the cultivar. Facón and Pampa cultivars had the highest number of infectious strains (41), whereas Bicentenario and Manantiales had the lowest (32 and 31, respectively).

Twenty-three strains were able to infect the four cultivars evaluated. The isolates obtained from nodules of the genus *Lathyrus* had a higher proportion of infective and effective nodules in the four cultivars evaluated (60%), followed by those from *Lens*, with 50%, and those from *Vicia*, with 29%, whereas the isolates from *Pisum*, with 8%. Soil isolates had 38% nodulation ability in the four cultivars.

Results from the growth chamber showed highly significant ($p<0.001$) variability in the $N°_{Nod}$ per plant (*i.e.* strain x cultivar), whereas the effects of strains on $Biom_{Total}$ were significant at $p=0.025$ (Additional material 2). $N°_{Nod}$ ranged from 76 to 1. D70 presented the highest $N°_{Nod}$ values, with an average of 43 nod pl^{-1}, followed by D191, with 35 nod pl^{-1}, and higher $N°_{Nod}$ than the rest. These responses were 97% and 67% higher, on average, at D70 and D191, respectively (Additional material 1). $Biom_{Total}$ ranged from 287 to 149 mg pl^{-1}, with D156, D155, and D191 showing the highest values (233-221 mg pl^{-1}), *i.e.* 6% higher than the $Biom_{Total}$ of D70 with the four cultivars (Additional material 1).

Based on these results, greenhouse experiments included strain D156 selected for biomass production ability, D191 for nodulation behavior, and D192 and D193 for fast initial growth (not shown) as compared to the reference strain D70.

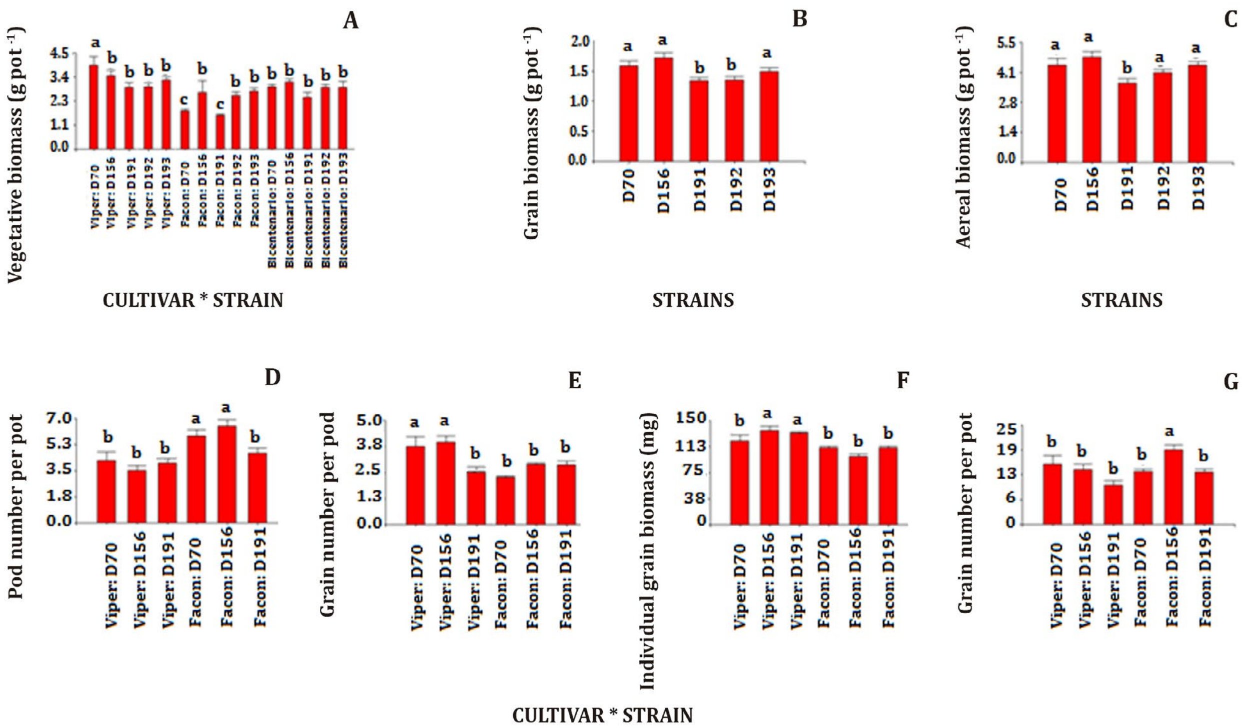
Greenhouse experiment

Strains D70, D156, D191, D192, and D193 were evaluated in the greenhouse experiment. At 56 DAP, a highly significant interaction between strain and cultivar was detected in all variables, except root biomass. Strain D192 had the best behavior in $Leng_{Root}$ (358 mm pl^{-1}) followed by D156 and D193 (322 mm pl^{-1}, on average) with cultivar Facón, with 28 and 15% differences in comparison to D70 and D191. D192 and D193 showed better $Leng_{Aerial}$ values, with 235 mm pl^{-1}, followed by D156 and D70 (206 mm pl^{-1}), all with cultivar Bicentenario, being 66% for 192 and D193 and 46% for D156 and D70. Instead, strain D193 had higher $Leng_{Plant}$ (515 mm pl^{-1}), followed by D192 (508 mm pl^{-1}) with Bicentenario and Facón. Likewise, strains D192 and D193 had higher $Biom_{Plant}$ (473 mg pl^{-1}) with Bicentenario, with significant differences with the rest of 55%. These strains also had the highest $Biom_{Aerial}$ values (271 mg pl^{-1}), followed by D156 and D70 (214 mg pl^{-1}), all with Bicentenario. In contrast, regarding nodule variables, D70 and D156 showed higher $N°_{Nod}$ (32 nod pl^{-1} on average) with Viper, 100% higher than the rest, as well as higher $Biom_{Nod}$ (9 mg pl^{-1} on average), 141% higher than the rest, and highest $Biom_{Nod\text{-}I}$ (0.44 mg nod^{-1} in average), 144% higher than the rest, all with Bicentenario.

At 78 DAP, a significant interaction between strain and cultivar was observed in the nodulation variables, except in individual nodule biomass. Strain D70 had the highest $N°_{Nod}$, together with strains D192 and D193, which were better associated with Bicentenario (109, 129, and 110 nod pot^{-1}, respectively). Also, strain D70 showed better $Biom_{Nod}$ with Viper (40 mg pot^{-1}) and Bicentenario (38 mg pot^{-1}), and the best $Biom_{Nod\text{-}I}$ (0.53 mg nod^{-1}) concerning other strains (75%). Differently, plant growth variables showed significant differences among strains. Strain D191 showed the lowest values of $Biom_{Aerial}$ (2.0 g pot^{-1}) and $Biom_{Root}$ (0.7 g pot^{-1}) with respect to the rest (40 and 39%, respectively).

At harvest (103 DAP), the five strains were evaluated for $Biom_{Veg}$, $Biom_{Grain}$, and $Biom_{Aerial.}$ Only $Biom_{Veg}$, showed cultivar and strain interaction. All plant yield components (*i.e.* $N°_{pod}$, $N°_{Grain}$, $N°_{Grain\text{-}pod}$ and $Biom_{Grain}$) showed significant interaction between both factors, although only measured in Facón and Viper with strains D70, D156, and D191. Strain D70 produced 3.9 g pot^{-1} of $Biom_{Veg}$ with Viper cultivar, significantly higher than the rest (45% on average), followed by D156 (3.5 g pot^{-1}) also with Viper cultivar. In contrast, strain D191 showed the worst performance (1.6 g pot^{-1}) with Facón (figure 1 A, page 6). Regarding $Biom_{Grain}$, D156, D70 and D193 had higher values (1.7, 1.6, and 1.5 g pot^{-1}, respectively) than D191 and D192 strains (figure 1B, page 6). Regarding $Biom_{Aerial}$, D191 (3.6 g pot^{-1}) showed significantly lower values (22%) than other strains (figure 1C, page 6). Considering yield components, strain D156, followed by D70, threw the best results compared to D191, except in $Biom_{Grain\text{-}I}$ where D191 had the second-best weight after D156. In addition, D156 stran had 6.5 pods pot^{-1}, followed by D70 strain (5.8 pods pot^{-1}), both with Facón cultivar having more pod number per pot ($N°_{Pod}$) than the rest (figure 1D, page 6). Regarding grain number per pod ($N°_{Grain\text{-}pod}$), D156 also performed better with Facón (4.0 grains pod^{-1}) and D70 with Viper (3.8 grains pod^{-1}) (figure 1E, page 6). Likewise, D156 followed by D191, had the highest individual grain biomasses ($Biom_{Grain\text{-}I}$) with 136 and 132 mg $grain^{-1}$ respectively, with Facón (figure 1F, page 6). D156 with Facón had higher $N°_{Grain}$ (18.8 grains pot^{-1}) than the rest (44%) (figure 1G, page 6).

Total N (%N_{upt}) and $\delta_{15}N$ (‰) contents of whole plants were determined at 92 DAP for Facón and Viper inoculated with D70, D156, and D191 (Additional material 3). D156 (2.2%) and D70 (2.1%) strains had higher biomass $_{upt}$%Nupt than D191 ($p<0.01$). The mean β value was -0.66‰, where D70 had lower depletion of $\delta_{15}N$ (-0.45‰), without differences with D156 (-0.55‰), being both over D191 (-0.88‰) with
a significance level of $p=0.06$ (Additional material 3).



A) vegetative weight (g), **B)** grain weight (g), **C)** aerial biomass (g), **D)** pod number, **E)** grain per pod, **F)** individual grain biomass (mg) and **G)** grain number per pod.

Different letters indicate significant differences between treatments at p≤0.05. Results shown in panel D correspond to cultivars Facón and Viper average values.

A) biomasa de grano (g), **B)** biomasa vegetativa (g) y **C)** biomasa aérea total (g), **D)** número de vainas por maceta, **E)** número de granos por vaina, **F)** biomasa individual del grano (mg) y **G)** número de granos por maceta.

Letras diferentes indican diferencias significativas entre tratamientos con valor p≤0,05. Los resultados mostrados en el panel D corresponden al promedio de los cultivares Facón y Viper.

Figure 1. Plant variables and yield components per pot at harvest (103 days after planting) as dry matter.

Figura 1. Variables de la planta y componentes del rendimiento por maceta a la cosecha (103 días después de la siembra).

Field experiment

In the field experiment, only nodule number had significant interaction between factors. Regarding strain behavior significant differences were found for $Biom_{Veg}$, $Biom_{Aerial}$, and $Biom_{Nod\text{-}I}$.

Strain D191 behaved differently than in the greenhouse regarding $Biom_{Veg}$, $Yield_{Grain}$, $Biom_{Aerial}$ and N_{upt}. Likewise, strain D191 strain had better behavior in $Biom_{Veg}$ (3.3 Mg ha^{-1}) and $Biom_{Aerial}$ (6.7 Mg ha^{-1}) than D156 (15% in both variables) and D70 (33 and 25%, respectively, table 1, page 7). In addition, D191 strain had higher N content in aerial biomass (212 kg N ha^{-1}) than D156 (8%) and D70 (16%). Average $Yield_{Grain}$ was 3.5 Mg ha^{-1}, $\%N_{FBN}$ was 70.5% and $N°_{FBN}$ was 139 kg N ha^{-1} (table 1, page 7). Instead, regarding $\%N_{upt}$, D156 (3.43%) and D70 (3.41%) showed higher values than D191 (8 and 7%, respectively), maintaining the same behavior as in the greenhouse (table 1, page 7). Strain D191 had the highest $N°_{Nod}$ per plant (10.5 nod pl^{-1}) differing 105% with the rest. Instead, D156 and D70 had highly significant differences (p≤0.01) in $Biom_{Nod\text{-}I}$ (2.5 and 1.7 mg nod^{-1}, respectively) with respect to D191 (162 and 85%, respectively), but with no differences in $Biom_{Nod}$ (table 1, page 7).

Table 1. Nodulation at 60 DAP, nitrogen plant variables at 95 DAP and plant harvest at 108 DAP.

Tabla 1. Nodulación a los 60 DDP, de las variables de nitrógeno en planta (95 DDS) y rendimiento de granos a cosecha (108 DDS).

	Plant variables (95 DAP)			Nodulation (60 DAP)	
Strain / Variable	**$Biom_{Veg}$ Mg ha^{-1}**	**$Yield_{Grain}$* Mg ha^{-1}**	**$Biom_{Aerial}$ Mg ha^{-1}**	**$Biom_{Nod}$ mg**	**$Biom_{Nod-I}$ mg**
D70	2.5 b	3.3 a	5.3 b	97.2 a	1.74 a
D156	2.9 b	3.3 a	5.8 b	104.3 a	2.46 a
D191	3.3 a	3.8 a	6.7 a	67.3 a	0.94 b
p-value	0.006	0.09	0.004	0.56	0.008
Nitrogen plant variables at harvest (108 DAP)					
Strain / Variable	**N_{upt} kg ha^{-1}**	**$\%N_{FBN}$ %**	**N_{FBN} kg ha^{-1}**	**$\%N_{above}$ %**	
D70	182 b	72 a	132 a	3.43 a	
D156	196 b	73 a	143 a	3.41 a	
D191	212 a	67 a	142 a	3.18 b	
p-value	0.03	0.25	0.51	0.02	

*Adjusted to 0.135 kg H_2O kg grain^{-1} grain moisture. Values followed by different letters in columns indicate significant differences at $p \leq 0.05$.

*Ajustado a una humedad de grano de 0,135 kg H_2O kg grano^{-1}. Los valores seguidos de letras diferentes en la columna indican diferencias significativas en $p \leq 0,05$.

Molecular characterization

Molecular identification of D70, D156, and D191 strains via partial sequencing of the 16S rRNA gene showed 100% identity with *Rhizobium leguminosarum*, and deposited in the same database under accession numbers KU933357, KX 066064, and KX346599, respectively. Sequences were then processed to obtain multiple alignments then concatenated for the construction of a phylogenetic tree. In turn, strains Az39 (*Azospirillum argentinense*) and E109 (*Bradyrhizobium japonicum*) were incorporated as members of other groups. Results of this analysis showed that D70 and D156 are more closely related than D191.

Discussion

Our results showed that nodulation ability of both native and naturalized isolates of rhizobia in field pea was similar to that observed by other authors (3, 8, 23, 38). For example, Ballard *et al.* (2004) found that 67% (22 of 33) of naturalized populations of rhizobia (applied to pea plants as soil suspensions) caused early and abundant nodulation in one cultivar of field pea, while our work found that 44-55% of native isolates of Argentina or introduced strains kept at the IMYZA-INTA collection were infective in four field pea cultivars. Likewise, Boivin *et al.* (2020b) observed that *Rhizobium leguminosarum* sv *viciae* are all potential symbionts of *Fabaceae* hosts (field pea, vetch, and lens) but display variable competitiveness to form root nodules. However, when *Fabaceae* legumes are exposed to natural soil bacterial populations, symbiotic efficiency is often suboptimal. In a recent study, Boivin *et al.* (2020a) determined that small genetic differences in *nodD* genes allowed observing a higher specificity degree among *R. leguminosarun* sv *vicia*e in pea, faba bean, and lentil. In the present study, we observed that rhizobia nodule isolates from *Lathyrus* sp and *Lens* sp were the most infective, followed by *Vicia* sp. Instead, isolates from pea plants did not ensure infection in all cultivars of the same species (*Pisum sativum*), since only 1 of 12 isolates/strains caused nodules in the four cultivars evaluated. Therefore, field pea cultivars could have genetic differences (2, 36), which, associated with the rhizobial genome, may influence competitiveness for nodulation as well as rhizosphere colonization, decisive steps in the competition for nodule occupancy by *R. leguminosarum* in soil populations (23).

The elite strain D70, isolated from *Vicia* sp, is most used in inoculant formulations. This strain has high nodulation ability and BNF contribution. Enrico *et al.* (2020) determined an average of 134 kg ha^{-1} of N_{FBN} in field pea (8 experiments, average). In this work, D70 had a contribution of 131 kg N ha^{-1}, while D156, also isolated from *Vicia* sp, showed a similar and better BNF behavior in both greenhouse and field experiments (*i.e.* 143 kg N ha^{-1}). Instead, the high nodulation ability observed in D191 (obtained from *Lens* sp) in the growth chamber, then resulted the least effective regarding yield variables in the greenhouse experiment. However, this strain performed better in the field experiment regarding biomass production (6.7 Mg ha^{-1}), grain yield (3.8 Mg ha^{-1}) and N absorbed (212 kg ha^{-1}), considering the other strains. Table 2 shows BNF contributions from different studies, indicating the selected strains had high N contribution. Likewise, D156 and D70 had better N content in biomass ($\%N_{upt}$), which, according to Laguerre *et al.* (2007), depends on BNF efficiency of rhizobia derived from a robust nodular system.

Table 2. Contribution of biological nitrogen fixation (BNF) on field pea.

Tabla 2. Contribución de la fijación biológica de nitrógeno (FBN) en arveja sobre experimentos de campo.

BNF contribution (%)	N derived from BNF kg N ha^{-1}	References
72	131	D70 strain (this work)
73	143	D156 strain (this work)
67	142	D191 strain (this work)
69-71	166-286	Kumar and Goh 2000
84	238	Voisin *et al.*, 2002
47-81	64-81	Hauggaard-Nielsen *et al.*, 2009
87-91	64-130	Abi-Ghanem *et al.*, 2011
37-85	45-106	Ruisi *et al.*, 2012
41-45	-	Keneni *et al.*, 2013
43-62	58-69	Hossain *et al.*, 2016
35-65	-	Yang *et al.*, 2017
55	63	Liu *et al.*, 2019
33-83	11-71	Enrico *et al.*, 2020

In the present study, strains D70 and D191 had high nodulation ability in controlled conditions (growth chamber and greenhouse). In contrast, both D156 and D70 had higher individual nodule biomass than D191, similar to controlled conditions. This behavior may be due to differences observed by the 16S genes concerning D70 or the larger number of specific genes that control nodulation competitiveness (8). This behavior can also be due to plant energetic regulation in association since, according to Rainbird *et al.* (1984), sustaining nodular system involves 22% of total energy, and nitrogenase activity (BNF) consumes an additional 52% (in soybean). Likewise, Ryle *et al*. (1986) determined that the energy consumption of white clover nodules was 22-27%. Therefore, nodule numbers adapt to plant possibility to maintain a symbiotic system benefiting yield.

Early growth effects in the growth chamber (D156) and greenhouse experiment (D156, D192, and D193 strains) could be associated with strain-dependant phytohormone effects, a fact not addressed in this study. Therefore, strains tending to increase auxins would be associated with stem and root elongation during post-emerging growth (6, 27, 33). In addition, cytokine balance in the early nodulation stages may be associated with a larger number of nodules. Also, final number of nodules may depend on ethylene production (4, 15). Strains D192, D193 and D156 stood out in stem and root elongation and nodule number and could have an adequate profile of phytohormones helping plant implantation. Then, at an advanced stage (78 DAP), the most relevant aspect would be symbiotic functionality, where the contribution of biological N translates into greater biomass (11, 34). Both strains D156 and D70 showed differences with D191, possibly due to symbiotic effectiveness. Differences were also observed in grain yield when the only N source was biological. Likewise, the only source of ^{15}N abundance in biomass resulted to be the biological source (β value), adjusting the contribution of BNF in field conditions. These results are consistent with those obtained by Unkovich *et al*. (1994) in clovers under different environments and with those by López-Bellido *et al*. (2010) in chickpea and faba beans between selected and natural soil strains. For non-different nodule structures (*i.e.* the same cultivar), differences in ^{15}N abundance in the plant would be associated with strain genotype, generating a distinctive behavior (23). This study confirms the importance of *Rhizobium* strains in ^{15}N isotopic discrimination in field pea, due to isolate origin, where adaptive or microevolutionary changes would be expected after genetic divergences (20, 28).

In our field experiment, aerial biomass ($Biom_{Aerial}$) and yield ($Yield_{Grain}$) had no interaction (same as in the greenhouse). Yields obtained from the field experiment (3.2 Mg ha^{-1} for D70, 3.3 for D156 Mg ha^{-1}, and 3.8 Mg ha^{-1} for D191; 3.5 Mg ha^{-1} on average) were higher than those obtained in the 2014 crop cycle by Prieto *et al.* (2015) (2.7 Mg ha^{-1}) and Enrico *et al.* (2020) (1.3 Mg ha^{-1}) in eight experiments performed in different environments of Argentina between 2012 and 2017. Unlike that observed in the greenhouse, inoculation with D191 showed the best behavior in aerial and vegetative biomass and nodule number per plant. In the field experiment, D156 and D70 had the same behavior on the specific variables derived from the *Rhizobium* strain, such as %N_{BNF} and/or the contribution of N_{BNF}, and maintained the efficiency demonstrated in the greenhouse (23). In this study, in the field experiment, we observed 70.5% (range 67 to 73%) of %N_{BNF} on average with 67% for D91, 72% for D70, and 73% for D156, all higher values than those established by Enrico *et al.* (2020), who found 59% inoculated with D70, but within the interquartile range obtained for field pea (33 to 83%).

The incorporation of a winter legume such as field pea (inoculated with efficient N-fixing strains) preceding a summer crop (*e.g.* maize) may constitute a valid agronomic management. Enrico *et al*. (2020) observed a positive effect on maize yield when the previous crop was inoculated (field pea with strain D70). Having rich in N and low C:N ratio stubble allowed rapid soil N availability. This higher availability of N considerably reduced the need for chemical nitrogen fertilization.

Conclusions

Most of the isolates that were infective and effective for *Pisum sativum* cultivars here evaluated came from species other than field pea.

Strain D70 maintained good behavior in the growth chamber, greenhouse, and field experiments concerning nodule, nitrogen, biomass, and yield variables, whereas, D156 had similar or better behavior in the greenhouse and field experiments. In contrast, D191 had only better behavior in the field experiment.

The β value calculation of ^{15}N natural abundance model to determine %N_{FBN} was key to adjusting N_{FBN} contribution. Determining differences in strain behavior results an essential requirement and would allow a more precise adjustment of N input.

Finally, strains selected for BNF efficiency should implicitly carry infectivity ability in all commercial cultivars. This will allow certainty in their recommendation for the formulation of inoculants. If new evaluations are required in different agroecological environments, strain D156 could be a good candidate for bacterial single-strain inoculants, as well as for a microbial consortium for *Pisum sativum*.

Supplemmentary material

Squematic figure of the dual-choice olfatometer utilized in choice test with stored grain pests:
https://drive.google.com/drive/folders/17vBzpdAKT_WP2l_SdnicLF_EH-puPzCy?usp=sharing

References

1. Abi-Ghanem, R.; Carpenter-Boggs, L.; Smith, J. L. 2011. Cultivar effects on nitrogen fixation in peas and lentils. Biology and Fertility of Soils. 47(1): 115-120.
2. Abi-Ghanem, R.; Bodah, E. T.; Wood, M.; Braunwart, K. 2013. Potential breeding for high nitrogen fixation in *Pisum sativum* L.: germplasm phenotypic characterization and genetic investigation. American Journal of Plant Sciences. 4(08): 1597.
3. Ballard, R. A.; Charman, N.; McInnes, A.; Davidson, J. A. 2004. Size, symbiotic effectiveness and genetic diversity of field pea rhizobia (*Rhizobium leguminosarum* bv. viciae) populations in South Australian soils. Soil Biology and Biochemistry. 36(8): 1347-1355.
4. Bartoli, C.; Boivin, S.; Marchetti, M.; Gris, C.; Gasciolli, V.; Gaston, M.; Lepetit, M. 2020. *Rhizobium leguminosarum* symbiovar viciae strains are natural wheat endophytes and can stimulate root development and colonization by arbuscular mycorrhizal fungi. hal-02967159. https://hal.inrae.fr/hal-02967159
5. Boddey, R. M.; Peoples, M. B.; Palmer, B.; Dart, P. J. 2000. Use of the 15N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutrient Cycling in Agroecosystems. 57: 235-270.
6. Boenel, M.; Fontenla, S.; Solans, M.; Mestre, M. C. 2023. Effect of yeast and mycorrhizae inoculation on tomato (*Solanum lycopersicum*) production under normal and water stress conditions. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 141-151. DOI: https://doi.org/10.48162/rev.39.116
7. Boivin, S.; Mahé, F.; Tancelin, M.; Tauzin, M.; Wielbo, J.; Mazurier, S.; Lepetit, M. 2020a. Genetic variation in host-specific competitiveness of the symbiont *Rhizobium leguminosarum* symbiovar viciae. DOI:10.22541/au.159237007.72934061
8. Boivin, S.; Ait Lahmidi, N.; Sherlock, D.; Bonhomme, M.; Dijon, D.; Heulin-Gotty, K.; Jensen, E. 2020b. Host-specific competitiveness to form nodules in *Rhizobium leguminosarum* symbiovar viciae. New Phytologist. 226(2): 555-568.
9. Collino, D. J.; Salvagiotti, F.; Perticari, A.; Piccinetti, C.; Ovando, G.; Urquiaga, S.; Racca, R. W. 2015. Biological nitrogen fixation in soybean in Argentina: relationships with crop, soil, and meteorological factors. Plant and Soil. 392: 239-252.
10. De Bortolli Pagnoncelli Jr., F.; Muzell Trezzi, M.; Bortolanza Pereira, P.; Rader, D. R.; Biedacha, R.; Galon, L.; Bresciani Machado, A. 2023. Volunteer soybean (*Glycine max*) interference in bean (*Phaseolus vulgaris*) crops: ethoxysulfuron and halosulfuron critical level of damage and selectivity. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 108-119. DOI: https://doi.org/10.48162/rev.39.113
11. Dejong, T. M.; Phillips, D. A. 1981. Nitrogen stress and apparent photosynthesis in symbiotically grown *Pisum sativum* L. Plant physiology. 68(2): 309-313.
12. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. W.; InfoStat versión 2018. Centro de Transferencia InfoStat, FCA. Universidad Nacional de Córdoba. Argentina. URL http://www.infostat.com.ar
13. Enrico, J. M.; Piccinetti, C. F.; Barraco, M. R.; Agosti, M. B.; Eclesia, R. P.; Salvagiotti, F. 2020. Biological nitrogen fixation in field pea and vetch: Response to inoculation and residual effect on maize in the Pampean region. European Journal of Agronomy. 115: 126016.
14. Galon, L.; do Amarante, L.; Favretto, E., L.; Cavaletti, D. C.; Henz Neto, O. D.; Brandler, D.; Senhori, V. M.; Concenço, G.; Stradioto Melo, T.; Aspiazú, I.; Muzzel Trezzi, M. 2022. Competitive ability of bean *Phaseolus vulgaris* cultivars with *Urochloa plantaginea*. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 117-131. DOI: https://doi.org/10.48162/rev.39.071
15. Gresshoff, P. M.; Lohar, D.; Chan, P. K.; Biswas, B.; Jiang, Q.; Reid, D.; Ferguson, B.; Stacey, G. 2009. Genetic of ethylene regulation of legume nodulation. Plant signaling & behavior. 4(9): 818-823.
16. Hauggaard-Nielsen, H.; Gooding, M.; Ambus, P.; Corre-Hellou, G.; Crozat, Y.; Dahlmann, C.; Dibet, A.; von Fragstein, P.; Pristeri, A.; Monti, M.; Jensen, E. S. 2009. Pea–barley intercropping for efficient symbiotic N2-fixation, soil N acquisition and use of other nutrients in European organic cropping systems. Field crops research. 113(1): 64-71.
17. Hoagland, D. R.; Arnon, D. I. 1938. The water culture method for growing plants without soil. Circular 347. Agricultural Experimental Station, University of California.
18. Hossain, Z.; Wang, X.; Hamel, C.; Knight, J. D.; Morrison, M. J.; Gan, Y. 2016. Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. Canadian journal of plant science. 97(1): 119-131.
19. Jantalia C. P.; Dos Santos, H. P.; Urquiaga, S.; Boddey, R. M.; Alves, B. J. R. 2008. Fluxes of nitrous oxide from soil under different crop rotations and tillage systems in the South of Brazil. Nutr Cycl Agroecosyst. 82: 161-173.
20. Jozefkowicz, C.; Brambilla, S.; Frare, R.; Stritzler, M.; Puente, M.; Piccinetti, C.; Ayub, N. 2017. Microevolution rather than large genome divergence determines the effectiveness of legume-rhizobia symbiotic interaction under field conditions. Journal of Molecular Evolution. 85: 79-83.
21. Keneni, G.; Assefa, F.; Imtiaz, M.; Bekele, E.; Regassa, H. 2011. Genetic diversity for attributes of biological nitrogen fixation in Abyssinian field pea (*Pisum sativum* var. Abyssinicum) germplasm accessions. Journal of Genetics and Breeding (under review).

22. Kumar, K.; Goh, K. M. 2000. Biological nitrogen fixation, accumulation of soil nitrogen and nitrogen balance for white clover (*Trifolium repens* L.) and field pea (*Pisum sativum* L.) grown for seed. Field Crops Research. 68(1): 49-59.
23. Laguerre, G.; Depret, G.; Bourion, V.; Duc, G. 2007. *Rhizobium leguminosarum* bv. viciae genotypes interact with pea plants in developmental responses of nodules, roots and shoots. New Phytologist. 176(3): 680-690.
24. Liu, L.; Knight, J. D.; Lemke, R. L.; Farrell, R. E. 2019. A side-by-side comparison of biological nitrogen fixation and yield of four legume crops. Plant and Soil. 442(1-2): 169-182.
25. López-Bellido, F. J.; López-Bellido, R. J.; Redondo, R.; López-Bellido, L. 2010. B value and isotopic fractionation in N 2 fixation by chickpea (*Cicer arietinum* L.) and faba bean (*Vicia faba* L.). Plant and soil. 337(1-2): 425-434.
26. Masson-Boivin, C.; Giraud, E.; Perret, X.; Batut, J. 2009. Establishing nitrogen-fixing symbiosis with legumes: how many rhizobium recipes? Trends in microbiology. 17(10): 458-466.
27. Mazur, A.; De Meyer, S. E.; Tian, R.; Wielbo, J.; Zebracki, K.; Seshadri, R.; Reddy, T. B. K.; Markowitz,V.; I vanova, N.; Pati, A.; Woyke, T.; C. Kyrpides, N.; Wayne, T. 2015. High-quality permanent draft genome sequence of *Rhizobium leguminosarum* bv viciae strain GB30; an effective microsymbiont of *Pisum sativum* growing in Poland. Standards in genomic sciences. 10(1): 36.
28. Mutch, L. A.; Young, J. P. W. 2004. Diversity and specificity of *Rhizobium leguminosarum* biovar viciae on wild and cultivated legumes. Molecular Ecology. 13(8): 2435-2444.
29. Prieto, G.; Amado, R.; Apella, C.; Avila, F.; Brassesco, R.; Buschittari, D.; Coletta, M.; Depaolo, J.; Espósito, A.; Fariña, L.; Figueroa, E.; Gordo, L.; López Tessore, M.; Maggio, J.; Manso, L.; Martínez, S.; Martínez, G.; Martins, L.; Pérez, G.; Vallejos, M.; Vita, E.; Vizgarra, O. 2015. Rendimiento de cultivares de Arveja (*Pisum sativum* L) en diferentes ambientes de la República Argentina. Campaña 2014/2015. Agricultores Federados Argentinos. N° 23: 71.
30. Rainbird, R. M.; Hitz, W. D.; Hardy, R. W. 1984. Experimental determination of the respiration associated with soybean/Rhizobium nitrogenase function, nodule maintenance, and total nodule nitrogen fixation. Plant physiology. 75(1): 49-53.
31. Ruisi, P.; Giambalvo, D.; Di Miceli, G.; Frenda, A. S.; Saia, S.; Amato, G. 2012. Tillage effects on yield and nitrogen fixation of legumes in Mediterranean conditions. Agronomy journal. 104(5):1459-1466.
32. Ryle, G. J. A.; Powell, C. E.; Gordon, A. J. 1986. Defoliation in white clover: nodule metabolism, nodule growth and maintenance, and nitrogenase functioning during growth and regrowth. Annals of botany. 57(2): 263-271.
33. Subramanian, S. 2013. Distinct hormone regulation of determinate and indeterminate nodule development in legumes. J Plant Biochem Physiol. 1(110): 2.
34. Unkovich, M. J.; Pate, J. S.; Sanford, P.; Armstrong, E. L. 1994. Potential precision of the δ15N natural abundance method in field estimates of nitrogen fixation by crop and pasture legumes in south-west Australia. Australian Journal of Agricultural Research. 45(1): 119-132.
35. Vincent, J. M. 1970. A manual for the practical study of the root nodule bacteria. In: IBP Handbook 15. Blackwell Scientific Publications. Oxford. UK.
36. Voisin, A. S.; Salon, C.; Munier-Jolain, N. G.; Ney, B. 2002. Quantitative effects of soil nitrate, growth potential and phenology on symbiotic nitrogen fixation of pea (*Pisum sativum* L.). Plant and soil. 243(1): 31-42.
37. Weisburg, W. G.; Barns, S. M.; Pelletier, D. A.; Lane, D. J. 1991. 16S ribosomal DNA amplification for phylogenetic study. Journal of bacteriology. 173(2): 697-703.
38. Wielbo, J.; Podleśna, A.; Kidaj, D.; Podleśny, J.; Skorupska, A. 2015. The diversity of pea microsymbionts in various types of soils and their effects on plant host productivity. Microbes and environments. ME14141.
39. Yang, C.; Bueckert, R.; Schoenau, J.; Diederichsen, A.; Zakeri, H.; Warkentin, T. 2017. Symbiosis of selected *Rhizobium leguminosarum* bv viciae strains with diverse pea genotypes: effects on biological nitrogen fixation. Canadian journal of microbiology. 63(11): 909-919.

Effects of geomorphology and distribution of water sources for livestock on the floristic composition and livestock receptivity of the Arid Chaco

Efecto de la geomorfología y la distribución de las fuentes de agua para el ganado en la composición florística y receptividad ganadera del Chaco Árido

Juan Antonio Scaglia [1, 2*], Daniel German Flores [1, 4], Raúl Tapia [2, 3, 4], Mariana Martinelli [2, 4]

Originales: *Recepción:* 07/11/2023 - *Aceptación:* 26/03/2024

Abstract

Livestock production in semi-arid areas is possible due to the presence of permanent water sources, which create a radial pattern of grazing intensity known as the piosphere. For this reason, we predicted that permanent water sources would negatively impact the ecological conditions of plant communities, leading to variations in livestock receptivity. To test this prediction, we compared grazing gradients in two geomorphological units, using distance to water sources as an indicator of accumulated livestock pressure. We assessed variations in the botanical composition of both areas by analysis of variance and principal components analysis. Additionally, we modeled the relationship between distance to water source and livestock receptivity. Our results revealed significant differences in the contribution of different species based on their distance to water sources. Notably, a non-linear regression model provided the best fit for the relationship between water source and livestock receptivity in both geomorphological units. These findings demonstrate that the distance to permanent water sources serves as a reliable indicator of accumulated livestock pressure in semi-arid regions like the study area.

Keywords
arid Chaco • natural grasslands • piosphere • geomorphology

1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).
* joposcaglia@gmail.com
2 Instituto Nacional de Tecnología Agropecuaria (INTA). Estacion Experimental Agropecuaria San Juan Calle 11 y Vidart 5427. Villa Aberastain. San Juan. Argentina.
3 Universidad Nacional de San Juan (UNSJ). Facultad de Ingenieria. Av. Lib. San Martín (Oeste) 1109. C. P. A. J5400ARL.
4 Universidad Nacional de San Juan (UNSJ). Facultad de Ciencias Exactas, Físicas y Naturales (FCEFyN). Cátedra de Manejo de Bosques y Pasturas Naturales. Gabinete de Geología Ambiental. Av. Ignacio de la Roza 590 (O). Complejo Universitario "Islas Malvinas". Rivadavia. C. P. A. J5402DCS. San Juan. Argentina.

Resumen

La producción de ganado en zonas semiáridas es posible debido a la existencia de fuentes de agua permanentes, lo que genera un patrón radial de intensidad de pastoreo llamada piosfera. Por tal motivo, nuestra predicción se basa en que las fuentes de agua permanente influyen negativamente en las condiciones ecológicas de las comunidades vegetales provocando diferentes receptividades ganaderas. Comparamos gradientes de pastoreo tomando la distancia a las fuentes de agua como indicador de presión ganadera acumulada en dos unidades geomorfológicas. La variación en la composición botánica de las diferentes áreas se realizó utilizando análisis de la varianza y un análisis de componentes principales. Se efectuó un modelo de la relación entre la distancia a la fuente de agua y la receptividad ganadera. Nuestros resultados mostraron diferencias significativas en la contribución de las diferentes especies en relación con la distancia a las fuentes de agua. El modelo de regresión no lineal fue el que mejor se ajustó entre la fuente de agua y la receptividad ganadera para ambas unidades geomorfológicas. La distancia a las fuentes de agua permanente es un buen indicador de la presión ganadera acumulada en regiones semiáridas como el área de estudio.

Palabras clave
Chaco árido • pastizales naturales • piosfera • geomorfología

Introduction

Like other arid and semi-arid regions, the Arid Chaco exhibits heterogeneous spatial patterns of degradation driven by the uneven distribution of drinking throughs (4, 19). The availability of permanent water sources is critical for livestock production in these areas. Consequently, a radial pattern of grazing intensity, known as 'piosphere,' develops around water sources. Analysis of piospheres allows to quantify the effects of radial attenuation of a disturbance on the system's condition (8).

Piospheres are areas around water sources that suffer from heavy grazing and trampling, making it difficult for vegetation to establish (degraded zones) (22). Management practices near these water bodies often involve continuous grazing without rest periods for the land, leading to changes in plant composition (10). As a result, plant communities near water sources are dominated by annual grasses and/or species with little grazing value (23).

Grassland dynamics can be conceptualized using a state-and-transition model (STM), which describes vegetation as existing in discrete states with transitions triggered by natural events or management practices (5, 38). Within an STM framework, each state represents a distinct plant community reflecting the current ecological conditions relative to a climax community. Transitions within a state are considered reversible, while transitions between states can be irreversible depending on disturbance severity (26, 36).

In interaction with livestock, geomorphology shapes the spatial patterns and dynamics of plant communities (27). In arid and semi-arid environments, water availability is the primary control of vegetation structure and function, surpassing even the influence of the physical and chemical characteristics of each geomorphological unit (24). Recent sedimentary environments, are particularly sensitive to the colonization strategies employed by vegetation given the specific arrangement and structures of their deposits (15).

Evaluating the impact of grazing on ecosystem integrity becomes difficult, especially in systems where the original condition is unknown given widespread and irreversible transformations of plant communities (10). Furthermore, when a system has surpassed a critical threshold and transitioned to an alternative stable state, experiments utilizing grazing exclusion methods may yield misleading interpretations (9).

An alternative approach to evaluate grazing is to interpret current vegetation assemblages in the context of accumulated livestock pressure and associated management practices. Proxy indicators such as proximity to water sources (1), livestock posts (32), and even specific plant growth forms or species abundance (13, 18) can be employed for this purpose. These approaches often provide solid information to inform management decisions, particularly when time constraints prevent reliance on long-term grazing trials (10).

This study investigates the impact of extensive livestock farming on botanical composition and livestock receptivity within distinct geomorphological units of the Arid Chaco region in San Juan, Argentina. We aim to contribute to the understanding of the ecological state of plant communities in semi-arid areas assessing livestock receptivity potential and informing sustainable management strategies. The study area exhibits diverse plant communities with varying physiognomies, shaped by the combined effects of livestock farming, forestry exploitation, and interactions with geomorphological processes.

Within this framework, our study hypothesizes that botanical composition within each geomorphological unit varies in response to distance from water sources. This prediction is based on permanent water sources negatively influencing the ecological states of plant communities, leading to differential livestock receptivity.

Materials and methods

Study area

The study area covers approximately 1000 km^2 within the Valle Fértil department, located between parallels 30°50' and 30°29' S and meridians 67°27' and 67°12' W. It is located east of the Sierras de Valle Fértil-La Huerta within the Bajo Oriental depression, bordering La Rioja to the east. This region represents a unique and characteristic expression of the Arid Chaco in San Juan, exhibiting distinctive physiographic, climatic, social, and productive features. Its climate is classified as arid (BWk) according to the Köppen-Geiger system (20), with an average annual temperature of 17.9°C. Precipitation falls within the 200-300 mm isohyets. The vegetation comprises an open forest dominated by *Aspidosperma quebracho blanco*, *Neltuma flexuosa*, and *Bulnesia retama*. A shrub layer rich in *Larrea divaricata* is present, while the herbaceous layer is well-represented by genera such as *Leptochloa*, *Setaria*, *Aristida*, and *Pappophorum*, among others.

Basin delimitation

A high-resolution (12.5-meter pixel size) Alos Palsar Digital Elevation Model (DEM) was used to delineate the basins. Flow direction derived from the DEM was employed to identify the basins associated with the main channels within the study area. GRASS GIS software, a free and open-source module linked to QGIS 3.14.15 (30), was used for the digital processing of the DEM.

Given the study area corresponds to a plain, vectors representing the mountain range were excluded from the analysis. Consequently, sampling was focused on the geomorphological units of the foothills (Piedemonte) and the river floodplain (Floodplain) (figure 1, page 15).

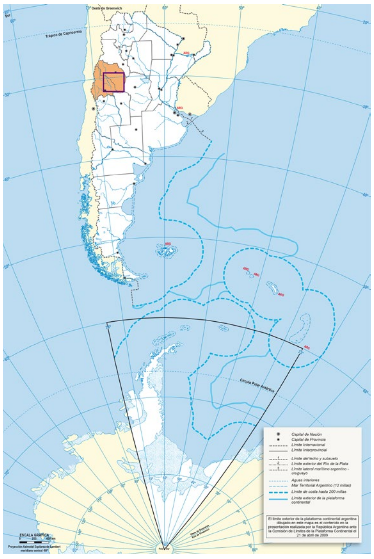
Location of livestock posts

The study area encompasses a diversity of fenced enclosures with varying surface areas. Each enclosure has a water source (dam, well, or drinker) for livestock located in the vicinity of the post. All sampled fields employed a continuous grazing regime, where cattle roam freely with unrestricted access to a central water source. During periods of extreme drought, ranchers are forced to either sell animals at a reduced price or pay to graze them in fields with superior forage availability.

This study adopts the methodology proposed by Cingolani *et al.* (2008), who emphasizes the value of distance to permanent water sources as an objective, measurable, and precise indicator for assessing the long-term effects of extensive grazing.

Establishment of distance ranges

Given the extensive grazing behavior of livestock in the Arid Chaco region (16), we categorized sampling locations according to their distance to water sources: close range (0-1000 m), intermediate range (1001-2000 m), and far range (>2001 m). This approach was applied within each geomorphological unit (Piedemonte and Floodplain). Thirty 50-meter transects were established within each unit, with readings taken at 50 cm intervals along the transects (figure 1, page 15). In the Piedemonte, ten transects were located close to water sources (within 1000 m, designated PMC), ten at intermediate distances (1001-2000 m, PMI), and ten at a far range (>2001 m, PML). Similarly, in the Floodplain, ten transects were placed near water sources (<1000 m, PLC), ten at intermediate distances (1001-2000 m, PLI), and ten at a far range (>2001 m, PLL) (figure 1).



Fuente/Source: IGN (Instituto Geográfico Nacional, República Argentina).

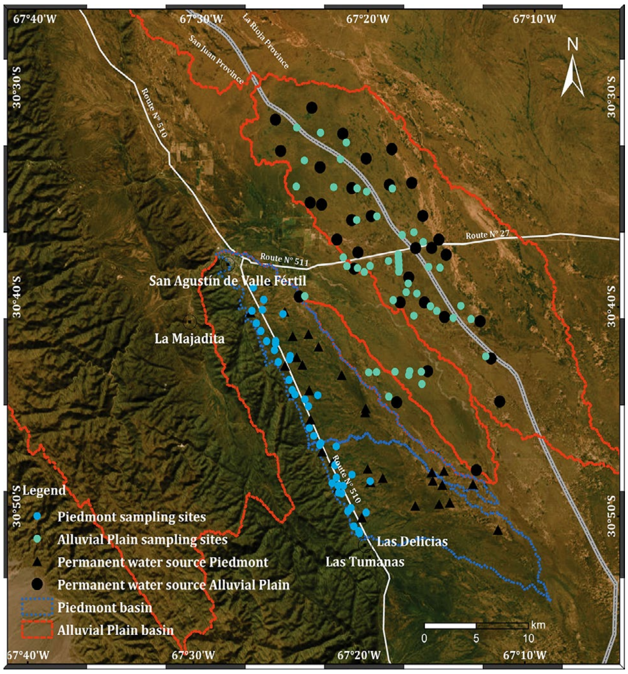


Figure 1. Location of study sites on the Arid Chaco phytogeographic province of San Juan and La Rioja, Argentina. Projection: Posgar 2007; Argentina/2; EPSG: 5344.

Figura 1. Ubicación de los sitios de estudio en la Provincia Fitogeográfica del Chaco Árido de la provincia de San Juan y La Rioja, Argentina. Proyección: Posgar 2007; Argentina/2; EPSG: 5344.

Determination of botanical composition

Botanical composition was quantified at peak growing season using the modified Point Quadrat method (28) in April and May, following the rainy season. The resulting data were then used to calculate percent coverage and specific contribution per contact (CSC) for each species.

$$CSC = \frac{C}{\sum C} * 100$$

where:
C = Contacts of a species
ΣC= Sum of the contacts of all species

Determination of field forage receptivity for bovine cattle

Livestock receptivity refers to the amount of forage required to sustain an Equivalent Cow Unit (EV). An EV is defined as a 400 kg cow that gestates and raises a calf to 6 months old (weighing 160 kg), including the forage consumed by the calf (Passera, C.P.Q.). One EV can be supported by 100 Pastoral Value (VP) units.

Plant species for calculating Pastoral Value (VP) were selected based on established criteria for determining specific quality indices (19, 29). Table 1 (page 16-19), presents their classification according to livestock preference: Preferential (P) - readily consumed species without selection; Good (G) - species initially rejected compared to Preferential ones; Regular (R) - species presenting some difficulty in consumption.

Table 1. Contribution of each species, total coverage, and diversity indices in each sector considered for sampling within each Geomorphological Unit separately.

Tabla 1. Aporte de cada especie, cobertura total e índices de diversidad en cada uno de los sectores considerados para el muestreo en cada Unidad Geomorfológica por separado.

	Geomorphological Units								
	Piedemonte				**Floodplain**				
	PML	**PMI**	**PMC**	**C**	**PLL**	**PLI**	**PLC**	**C**	**S**
Species	**Perennial Grasses**								
Leptochloa pluriflora	11.03c	-	2.48b	0.54	15.25d	10.36d	-	0.63	P
Gouinia paraguayensis	2.49b	0.63a	0.52a	0.54	1.34e	2.45d	2.78d	-0.48	P
Setaria lachnea	5.23b	5.99b	0.16c	0.5	1.21d	0.54d	-	0.62	P
Setaria leucopila	5.93b	6.73b	3.84a	0.32	6.96d	6.00d	-	0.49	P
Setaria cordobensis	0.64	-	-	0.44	-	-	-	-	P
Digitaria californica	1.71b	2.16b	0.47a	0.41	0.60d		0.52d	0.19	P
Cenchrus ciliaris	-	0.16	-	0.04	-	-	-	-	P
Cottea pappophoroides	1.63b	0.68a	1.60b	-0.09	-	-	-	-	G
Setaria hunzikeri	1.9	-	-	0.55	-	-	-	-	P
Pappophorum philippianum	1.32a	4.85b	11.12b	-0.48	-	-	-	-	P
Neobouteloua lophostachia	0.87a	2.41b	4.06b	-0.43	0.92d	2.34d	10.75e	-0.59	R
Leptochloa crinita	16.34a	1.73b	0.53b	0.56	1.13d	1.84d	-	0.33	P
Leptochloa dubia	0.34	-	-	0.56	-	-	-	-	P
Chloris parvispicula	0.79	-	-	0.34	-	-	-	-	P
Aristida medocina	-	-	0.18	-0.58	11.14d	1.14e	2.78e	0.71	P
Setaria sp	-	-	-		0.81	-	-	0.65	P
Sporobolus pyramidatus	0.17a	0.31a	-	0.29	0.40d	-	1.08d	-0.3	G

Different letters indicate significant differences (P < 0.05). The column labeled C represents the correlation of each species, total coverage, and diversity indices with respect to the distance from water sources. Significant correlations (P < 0.05) are indicated by grayed cells.

Letras diferentes indican diferencias significativas (P<0,05). La columna indicada con C representa la correlación de cada especie, cobertura total e índices de diversidad, respecto de la distancia con las fuentes de agua. Las correlaciones significativas (P<0,05) se indican con los casilleros pintados de gris.

Geomorphological Units									
	Piedemonte				**Floodplain**				
	PML	**PMI**	**PMC**	**C**	**PLL**	**PLI**	**PLC**	**C**	**S**
Annual Grasses									
Bouteloua aristoides	7.21a	19.85b	31.13b	-0.55	-	-	-	-	R
Eragrostis mexicana var. virescens	0.84b	-	0.38b	0.34	-	-	-	-	G
Aristida adscensionis	0.13a	0.81a	1.28a	-0.49	2.36d	-	0.36d	0.38	R
Tripogon spicatus	2.03	-	-	0.55	-	-	-	-	R
Shrubbery									
Larrea divaricata	9.05a	17.86b	20.26b	-0.75	30.82d	33.97d	55.71e	-0.74	-
Lippia turbinata	0.48b	-	0.38b	0.16	-	-	-	-	-
Mimozyganthus carinatus	1.30a	5.92a	1.26a	0.05	4.54	-	-	-	R
Cordobia argentea	0.60ab	0.68b	0.16a	0.43	5.60d	16.51e	8.65d	-0.24	G
Capparis atamisquea	0.48	-	-	0.55	-	-	-	-	R
Senna aphyla	1.95a	7.49b	0.49c	0.17	0.46d	5.65e	1.20d	-0.1	R
Pithecoctenium cynanchoides	0.67	-	-	0.56	-	-	-	-	-
Lantana fucata	1.74a	0.54b	-	0.67	-	-	-	-	-
Senegalia gilliesii	0.12a	-	0.57a	-0.35	-	-	-	-	R
Lippia integrifolia	1.00a	-	1.88a	-0.24	1.38	-	-	0.56	-
Ximenia americana	-	0.51a	0.65a	-0.58	-	3.57	-	-0.02	-
Aloysia gratissima	1.05a	0.16b	-	0.72	-	-	-	-	-
Condalia microphylla	0.51	-	-	0.56	-	0.54	-	-0.06	R
Tillandsia sp	0.13a	-	0.16a	-0.18	-	-	-	-	R
Lantana grisebachii	-	2.93a	5.00a	-0.67	-	-	-	-	-
Strombocarpa torquata		2.38a	0.47b	-0.1	0.44d	5.66e	8.34e	-0.74	R
Salvia gilliesii	-	-	-	-	0.23	-	-	0.56	-
Cereus aethiops	-	-	-	-	0.23	-	-	0.56	-
Ephedra triandra	-	-	-	-	0.21	-	-	0.45	G
Lycium chilensis var. filifolium	1.02c	0.33a	0.34a	0.53	0.23	-	-	0.56	R
Justicia gilliesii	2.89a	-	0.38b	0.55	-	-	-	-	G

Different letters indicate significant differences (P<0.05). The column labeled C represents the correlation of each species, total coverage, and diversity indices with respect to the distance from water sources. Significant correlations (P<0.05) are indicated by grayed cells.

Letras diferentes indican diferencias significativas (P<0,05). La columna indicada con C representa la correlación de cada especie, cobertura total e índices de diversidad, respecto de la distancia con las fuentes de agua. Las correlaciones significativas (P<0,05) se indican con los casilleros pintados de gris.

	Geomorphological Units								
	Piedemonte				Floodplain				
	PML	PMI	PMC	C	PLL	PLI	PLC	C	S
Mimozyganthus carinatus	1.30a	5.92a	1.26a	0.05	4.54	-	-	0.66	R
Lantana xenica	-	-	0.18	-0.58	-	0.31d	3.31e	-0.68	-
Tricomaria usillo	-	-	0.18	-0.58	1.38d	3.92e	0.96d	0.07	G
Forestry									
Parkinsonia praecox var praecox	3.53a	2.55a	3.65a	3.00E-03	0.42e	1.92d	-	0.06	R
Celtis tala	0.66a	0.16a	-	0.36	-	-	-	-	R
Neltuma flexuosa	8.27a	0.33b	1.30b	0.53	2.11d	2.01d	3.13d	-0.26	G
Geoffroea decorticans	-	-	-		6.75d	1.18d	0.52e	0.81	G
A quebracho blanco	-	2.44b	0.19b	0.01	-	-	-	-	-
Bulnesia retama	0.51a	4.39b	0.16a	0.06	-	-	-	-	-
Dicotyledonous herbaceous plants									
Bidens subalternans	0.29b	-	0.38b	-0.06	-	-	-	-	-
Pseudabutilon virgatum	0.25a	0.18a	0.95a	-0.37	-	-	-	-	-
Zinnia pruviana	0.25a	-	0.19a	0.1	-	-	-	-	-
Talinum polygaloides	-	-	0.18	-0.58	-	-	-	-	-
Gaya parvifolia	0.46a	0.16a	-	0.72	0.69d	-	0.52d	0.14	-
Amaranthus standleyanus	0.26	-	-	0.34	-	-	-	-	-
Gomphrena pulchella	0.34a	-	0.19a	0.27	-	-	-	-	-
Tribulus terrestris	0.12	-	-	0.56	-	-	-	-	-
Parthenium hysterophorus	0.13a	0.18a	-	0.25	-	-	-	-	-
Euphorbia catamarcensis	0.67	-	-	0.56	-	-	-	-	-
Sphaeralcea miniata	0.56a	1.88a	1.90a	-0.29	-	-	-	-	-
Allionia incarnata	0.13a	1.93b	1.14ab	-0.27	-	-	-	-	-
Heliotropium mendocinum	-	0.18	-	0.03	-	-	-	-	-
Porophyllum lanceolatum	-	0.16	-	0.04	-	-	-	-	-
Portulaca grandiflora	-	-	0.18	-0.58	-	-	-	-	-
Solanum elaeagnifolium	-	-	0.18	-0.58	-	-	0.52	-0.5	-

Different letters indicate significant differences ($P<0.05$). The column labeled C represents the correlation of each species, total coverage, and diversity indices with respect to the distance from water sources. Significant correlations ($P<0.05$) are indicated by grayed cells.

Letras diferentes indican diferencias significativas (P<0,05). La columna indicada con C representa la correlación de cada especie, cobertura total e índices de diversidad, respecto de la distancia con las fuentes de agua. Las correlaciones significativas (P<0,05) se indican con los casilleros pintados de gris.

Different letters indicate significant differences (P<0.05). The column labeled C represents the correlation of each species, total coverage, and diversity indices with respect to the distance from water sources. Significant correlations (P<0.05) are indicated by grayed cells.

Letras diferentes indican diferencias significativas (P<0,05). La columna indicada con C representa la correlación de cada especie, cobertura total e índices de diversidad, respecto de la distancia con las fuentes de agua. Las correlaciones significativas (P<0,05) se indican con los casilleros pintados de gris.

	Geomorphological Units								
	Piedemonte				Floodplain				
	PML	PMI	PMC	C	PLL	PLI	PLC	C	S
Diversity Coverage and Indexes									
Coverage	94.89a	88.96b	70.37c	-0.21	73.14d	67.78d	53.45e	0.63	
Shannon - Weaver	2.92a	2.54b	2.23c	0.68	2.21d	2.07d	1.58e	0.78	
Simpson	0.08a	0.12a	0.2b	-0.57	0.16c	0.19c	0.34d	-0.73	

The Pastoral Value (VP) is calculated by the following formula:

$$VP = 0.1 * (CSC * Is)\, Cf$$

where:
0.1 = constant coefficient
CSC = Specific Contribution per Contact
Cf = Forage Coverage
Is (Specific Quality Index) = ranging from 1 to 10, representing the classification of species based on their suitability and potential as forage.

Statistical analysis

Variation in botanical composition across different areas was explored using principal component analysis (PCA) based on a correlation matrix among all present species and their respective contact-specific contributions (CSC) within the community.

The influence of distance to water sources on botanical composition, diversity (Shannon-Weaver and Simpson), and total cover was assessed using analysis of variance (ANOVA) with a completely randomized design. Separate ANOVAs were conducted for each geomorphological unit (Piedemonte and Floodplain), treating distance categories (PMC, PMI, PML and PLC, PLI, PLL) as fixed effects. Additionally, a Pearson correlation analysis was performed to explore relationships between plant species, diversity indices, and total coverage. Data were square root transformed to address non-homoscedasticity and normality violations. Means were compared using Tukey's test. Data results are presented using untransformed means for ease of interpretation.

Visual exploration of the data involved plotting livestock receptivity against distance to water sources for each geomorphological unit. This analysis revealed a negative correlation up to a certain distance, followed by a plateau effect. Consequently, non-linear models were fitted for each unit to account for this observed pattern (12).

Results

Botanical composition

A total of 26 families were recorded, with *Poaceae* being the best-represented family, comprising 22 species. *Fabaceae*, *Verbenaceae*, and *Asteraceae* followed in abundance, with 7, 6, and 4 species, respectively.

The Piedemonte plant community is dominated by *Leptochloa crinita*. Additionally, *Leptochloa pluriflora*, *Bouteloua aristoides*, *Setaria leucopila*, *Setaria lachnea*, and *Gouinia paraguayensis* are present in PML sectors. In PMI and PMC sectors, the *Bouteloua aristoides* contribution increases, while the contributions of other grass species (except *Setaria leucopila* and *Setaria lachnea*, which maintain their presence in the PMI sector) decrease.

The Piedemonte shrub layer is dominated by *Larrea divaricata*, accompanied by *Mimosiganthus carinatus,* and *Senna aphylla*. The forest layer exhibits a distinct zonation: *Prosopis flexuosa* dominates in PML sectors, *Bulnesia retama* in PMI sectors, and *Aspidosperma quebracho blanco* occurs at low densities across all sectors.

Within the Floodplain, shrub communities dominate the vegetation structure. *Larrea divaricata* is the most abundant species, with accompanying species *Cordobia argentea* and *Mimozyganthus carinatus* in PLL sectors. In PLI sectors, shrub species with increased contributions include *Cordobia argentea*, *Senna aphyla*, and *Prosopis torquata*. Similarly, *Larrea divaricata* shows a significant increase in contribution within PLC sectors, accompanied by *Prosopis torquata*, *Lantana xenica*, and *Cordobia argentea*.

Following the shrub layer, grasses contribute significantly to the Floodplain community. *Aristida mendocina* and *Leptochloa pluriflora* are the most prominent grasses, with *Setaria leucopila* present to a lesser extent in PLL sectors. However, in PLC sectors, *Leptochloa pluriflora* disappears, and *Aristida mendocina* abundance declines significantly. Conversely, *Neobouteloua lophostachia* becomes the dominant grass species.

The forest layer plays a minor role in the Floodplain, with *Geoffroea decorticans* being the most prevalent species in PLL sectors, while *Prosopis flexuosa* dominates in PLI and PLC sectors (table 1, page 16-19).

Across both geomorphological units, total coverage and the Shannon-Weaver diversity index exhibit a significant rise with increasing distance from water sources. Conversely, Simpson's dominance index shows a significant positive correlation with proximity to water sources (table 1, page 16-19).

Principal Component Analysis (PCA) revealed a variation pattern in botanical composition across the sampled geomorphological units. The first two principal components explained 61% of the total species variance, effectively separating sectors within the Floodplain from those in the Piedmont. Within the Piedmont, sectors located close and halfway to water sources (PMC and PMI) were distinct from those situated further away (PML) (figure 2).

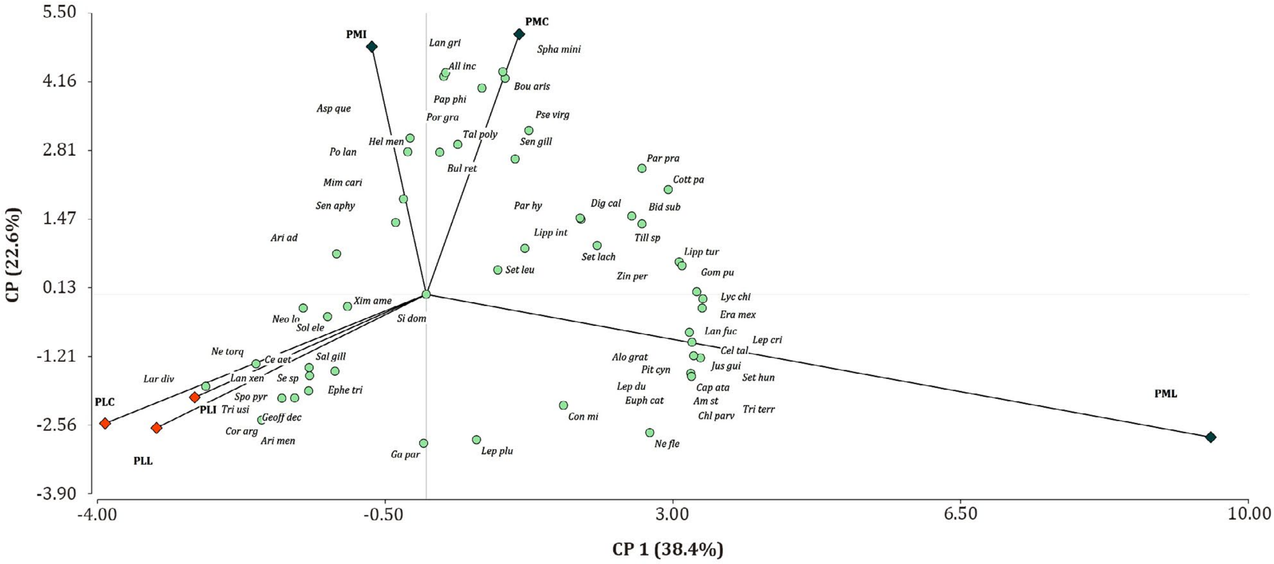


Figure 2. Principal Component Analysis (PCA) ordination diagram of sampling sectors (PMC, PMI, PML, PLC, PLI, PLL) and associated plant species.

Figura 2. Diagrama de la ordenación del Análisis de Componentes Principales (ACP) de los sectores seleccionados para los muestreos (PMC, PMI, PML, PLC, PLI, PLL) y las especies asociadas.

Within the alluvial plain, CP1 and CP2 displayed the most negative values for *L. divaricata* (Lar div), *C. argentea* (Cor arg), *T. usillo* (Tri usi), and *G. paraguayensis* (Ga par). In contrast, within the floodplain and specifically PML sectors, CP1 exhibited the most positive values for *J. gilliesii* (Jus gui), *L. chilensis var. filifolium* (Lyc chi), *S. cordobensis* (Set cor), *L. crinita* (Lep cri), *C. tala* (Cel tal), *N. flexuosa* (Ne fle), and *L. pluriflora* (Lep plu). Similarly, positive values in CP2 were associated with PMI and PMC sectors for *B. aristoides* (Bou aris), *A. quebracho blanco* (Asp que), *S. miniata* (Spha mini), *A. incarnata* (All inc), *L. grisebachii* (Lan gri), *P. philippianum* (Pap phi), *H. mendocinum* (Hel men), and *P. lanceolatum* (Po lan) (figure 2, page 20).

Livestock receptivity and distance to water sources

Non-linear regression analysis proved the best fit between livestock receptivity (Ha.EV^{-1}) and distance to water sources (DP), for both the Piedemonte (PM) and Floodplain (PL) units (figure 3).

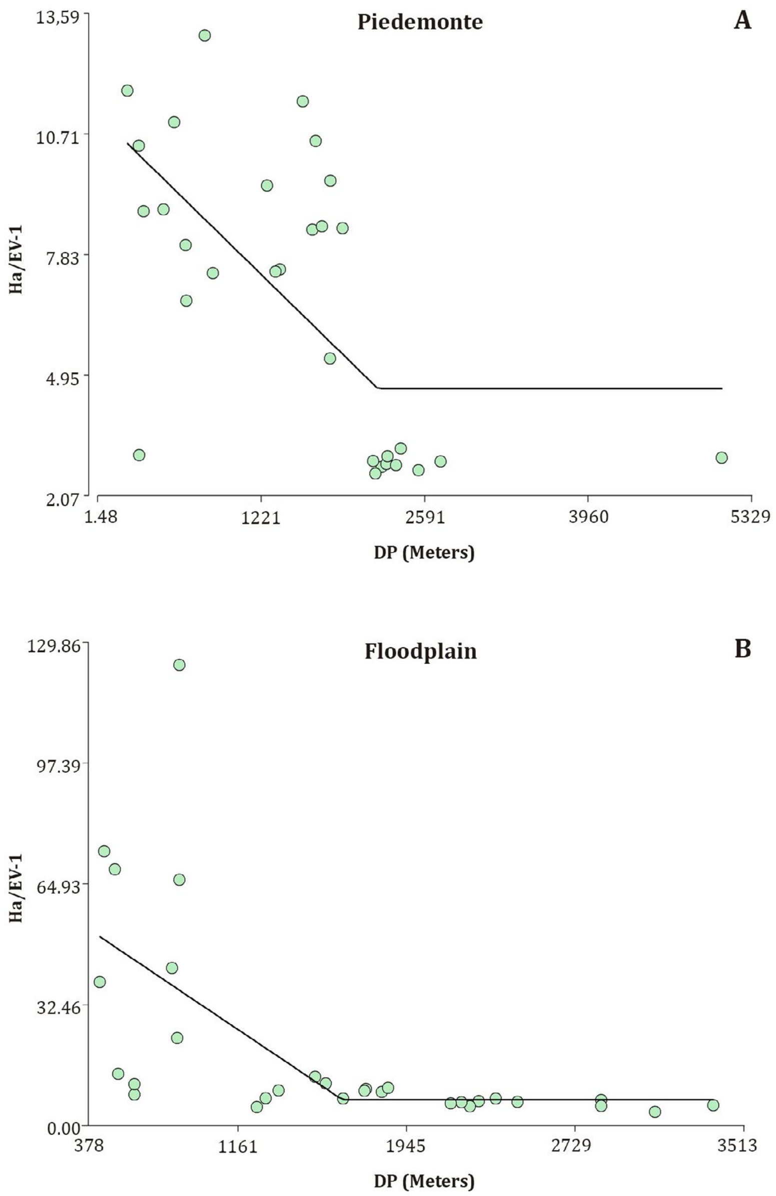


Figure 3. Non-linear regression model between livestock receptivity (Ha.EV^{-1}) and distance to water source (DP) in the Piedemonte (A) and Floodplain (B).

Figura 3. Modelo de regresión no lineal entre la receptividad ganadera (Ha.EV^{-1}) y la distancia a la fuente de agua (DP) en el Piedemonte (A) y en la Planicie de inundación (B).

Discussion

Our findings reveal changes in floristic composition across both geomorphological units, with distance to water sources playing a significant role (figure 2, page 20 and table 1, page 16-19). The study area is characterized by fenced fields of varying sizes and shapes, along with an irregular distribution of watering holes, dams, and/or livestock shelters. This heterogeneity contributes to a highly diverse botanical composition within plant communities. Szymañski *et al.* (2022) attribute similar variations primarily to livestock management practices. Specifically, our results indicate the presence of plant communities dominated by perennial forage grasses and others dominated by non-forage annual grasses. In such cases, the potential for transition towards improved ecological conditions exists due to the presence of seed sources from desirable species, facilitating the natural rehabilitation of nearby degraded areas (10).

The results align with established patterns of overgrazing documented by Briske *et al.* (2006), where plant communities near water sources (PMC and PLC) are dominated by annual grasses and/or non-forage species (table 1, page 16-19). While grazing responses vary among species, when grazing pressure alters biotic structures and interactions changes in species coverage can be attributed to underlying biotic mechanisms of grazing impact. Several studies support this notion, demonstrating that grazing pressure favors plant traits associated with rapid growth, regeneration, annual life cycles, and a ruderal strategy (11, 14, 32).

Across the geomorphological units, perennial grasses exhibited a significant trend of increased colonization away from water sources (table 1, page 16-19). Conversely, annual grasses and species with low forage value showed the opposite pattern. This aligns with established knowledge on grazing pressure inducing species turnover, favoring some species while hindering others (10). Briske *et al.* (2006), further suggests that heterogeneous livestock use is common in homogeneous landscapes (*i.e.*, within a single geomorphological unit). This heterogeneity contributes to an increase in landscape physiognomic diversity. However, increased livestock density can lead to a decline in unused areas (37). While maintaining some level of unused sites is important for overall community coexistence (10), excessively large paddocks with few water sources or shelters may allow even high livestock densities to coexist with unused areas (21, 26).

Total coverage and Shannon-Weaver diversity in the geomorphological units exhibited a significant decrease near water sources (table 1, page 16-19). Conversely, Simpson's dominance index shows a positive trend in these areas. This pattern aligns with observations in other semi-arid African grasslands, where grazing-sensitive forage species are replaced by those more resistant to grazing. However, this replacement may not fully compensate for diversity losses, potentially leading to environments dominated by a few resilient species. In this context, grazing regimes exceeding the historical range experienced by the ecosystem are likely to induce a significant decline in overall diversity (16).

The observed variations in floristic composition may be explained by mechanisms of resilience to harsh conditions proposed by Cingolani *et al.* (2008) for arid and semi-arid grasslands. These mechanisms, such as reduced sprouting and production of long-lived seeds, can confer some resistance to short periods of intense grazing. However, this resilience is insufficient to withstand continuous grazing for extended periods, particularly under moderate to high grazing pressure. A study evaluating intensity versus grazing strategies in perennial forage grasses of the Arid Chaco region found that while increased defoliation intensity may maximize short-term biomass yield, it negatively impacts long-term sustainability and produces detrimental residual effects on grasslands (31). Importantly, the study identified continuous grazing combined with high defoliation intensity as the least sustainable management practice for this ecosystem (31).

In these systems, continuous grazing by introduced herbivores without proper stocking management is highly likely to cause widespread extinctions and/or significant declines of specific plant species (10, 21, 25). However, a contrasting recovery trajectory may emerge when degradation and/or extinction of desirable species occurs homogenously across large areas (several square kilometers) and grazing pressure is subsequently relieved (10, 21). An alternative stability perspective for degraded ecosystems emphasizes persistence relative to the livelihood needs of ecosystem users. If the timeframe for ecosystem recovery surpasses user tolerance, typically a few years to a decade, the degraded state can be considered persistent from a practical viewpoint. Therefore, a 'persistent decline' of an ecosystem service may not always be associated with a critical threshold and a shift to a new ecological state. Slow recovery within a single stability domain can also lead to persistent declines (23).

Livestock receptivity values in this study range from 47.02 to 2.86 $Ha.EV^{-1}$ (figure 3, page 21). These values are consistent with observations from other arid and semi-arid regions in Patagonia (23), Puna, Monte, and the driest parts of the Arid Chaco, where reported livestock receptivities typically exceed 6 $Ha.EV^{-1}$ (3). Nevertheless, the value of 8.92 $Ha.EV^{-1}$ measured in the PMC sector deviates from historical data for degraded foothills, which reported values around 24 $Ha.EV^{-1}$ (33). This discrepancy may be attributed to interannual precipitation variations, known to cause substantial differences (>300%) in grassland productivity within degraded areas of the Arid Chaco (11).

Despite using the same model for both geomorphological units, the estimated values and rates of change differed (figure 3, page 21). These non-linear relationships align with the findings of Sasaki *et al.* (2008), who highlight the non-linear nature of ecological patterns and processes in response to grazing pressure. Furthermore, the estimated values from our models are consistent with other studies conducted in the region (2, 7, 11). Additionally, the observed spatial patterns of vegetation change correspond to documented livestock grazing behavior in the Arid Chaco (16). This distribution pattern of livestock receptivity informs spatial dynamics within grazing plots, allowing for the establishment of criteria for plot size and water source distribution.

Conclusions

Across both geomorphological units, distance to water sources significantly influences floristic composition, which in turn affects livestock receptivity. Areas closer to water sources are dominated by annual grasses and/or non-forage species, resulting in lower livestock receptivity. Conversely, areas farther from water sources are dominated by perennial grasses with higher forage value, leading to increased livestock receptivity.

A long history of domestic grazing has shaped the heterogeneity of plant communities, influencing species diversity and dominance. This translates to a landscape with varying ecological conditions (good, fair, and bad) distributed in different proportions. Quantifying the extent of each condition is crucial for optimizing water source distribution and achieving biologically and economically sustainable grazing management strategies. Notably, non-linear models provided the best fit between distance to water sources and livestock receptivity for both geomorphological units under study (figure 3, page 21).

References

1. Adler, P. B.; Milchunas, D. G.; Sala, O. E.; Burke, I. C.; Lauenroth, W. K. 2005. Plant traits and ecosystem effects of grazing: comparison of the American sagebrush steppe and the Patagonian steppe. Ecological Applications. 15 (2): 774-792.

2. Blanco, L. J.; Ferrando, C. A.; Biurrun, F. N. 2009. Detección remota de patrones espaciales y temporales de vegetación en dos sistemas de pastoreo. *Ecología y manejo* de pastizales. 62(5): 445-451.

3. Blanco, L. J.; Durante, M.; Ferrante, D.; Quiroga, R. E.; Demaria, M. R.; Di Bella, C. M. 2019. Red nacional de monitoreo de pastizales naturales de Argentina: productividad forrajera de la vegetación extrapampeana. Revista de Investigaciones Agropecuarias. 45(1): 89-108.

4. Blanco, L. B.; Aguilera, M. O.; Paruelo, J. M.; Biurrum, F. N. 2008. Grazing effect on NDVI acros san aridity gradient in Argentina. Journal of Arid Environments. 72: 764-776.

5.Briske, D. D. 2017. Rangeland systems processes, management and challenges. Springer. 10.1007/978-3-319-46709-2

6. Briske, D. D.; Fuhlendorf, S. D.; Smeins, F. E. 2006. A unified framework for assessment and application of ecological thresholds. Rangeland Ecology and Management. 59: 225-236.

7. Calella, H. F.; Corzo, R. R.; Gómez, J. C.; Reynoso, A. A.; Zalazar, A.; Murúa, S.; Ricarte, A. 2006. El Chaco árido de La Rioja: vegetación y suelos. Pastizales naturales. Ediciones INTA. Buenos Aires. Argentina.

8. Chillo, V.; Ojeda, R. A. 2014. Disentangling ecosystem responses to livestock grazing in drylands. Agriculture, Ecosystems and the Environment. 197: 271-277.

9. Cingolani, A. M.; Noy-Meir, I.; Diaz, S. 2005. Grazing effects on rangeland diversity: diversity-intensity and state and transition models. Ecological Applications. 15: 757-773.
10. Cingolani, A. M.; Renison, D.; Tecco, P. A.; Gurvich, D. E.; Cabido, M. 2008. Predicting cover types in a mountain range with long evolutionary grazing history: a GIS approach. Journal of Biogeography. 35(3): 538-551.
11. Díaz R., O. 2007. Utilización de pastizales naturales. Encuentro Grupo Editor. Córdoba, Argentina.
12. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. W. 2020. InfoStat versión 2020. Centro de Transferencia InfoStat, FCA. Universidad Nacional de Córdoba. Córdoba. Argentina. http://www.infostat.com.ar
13. Dyksterhuis, E. J. 1949. Condition and management of rangeland based on quantitative ecology. Journal of Range Management. 41: 450-459.
14. Espinoza, J. J. O.; Ayala, C. C.; Castillón, E. E.; Saldivar, F. G.; Sauceda, J. U.; Jurado, E.; Hernández, E. O. 2017. Livestock effect on floristic composition and vegetation structure of two desert scrublands in northwest Coahuila, Mexico. The southwestern naturalist. 62(2): 138-145.
15. Flores, D. G.; Suvires, G.; Dalmasso, A. 2015. El análisis geomorfológico como base para el estudio de la vegetación nativa: Sierra Chica de Zonda, Precordillera Oriental de Argentina. Cuadernos de Investigación Geográfica. 41(2): 427-444.
16. Hanke, W.; Böhner, J.; Dreber, N.; Jürgens, N.; Schmiedel, U.; Wesuls, D.; Dengler, J; 2014. The impact of livestock grazing on plant diversity: an analysis across dryland ecosystems and scales in southern Africa. Ecological Applications. 24(5): 1188-1203.
17. Herrera Conegliano, O. A. 2018. Comportamiento en pastoreo del ganado bovino Criollo Argentino y Aberdeen Angus ecotipo riojano, en pastizales naturales del Chaco Árido. Doctoral dissertation, Facultad de Ciencias Agrarias. Universidad Nacional de Mar del Plata.
18. Jauffret, S.; Lavorel, S. 2003. Are plant functional types relevant to describe degradation in arid, southern Tunisian steppes? Journal of Vegetation Science. 14(3): 399-408.
19. Karlin, M. S. 2013. Relaciones suelo-planta en el ecosistema Salinas Grandes, Provincia de Catamarca (Argentina). Doctoral thesis. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba.
20. Koppen, W. 1936. Das geographische System de Klimate. *Handbuch der klimatologie. Borntraeger. Berlín. Bd. 1.C.*
21. Landsberg, J.; James, C. D.; Morton, S. R.; Müller, W. J.; Stol, J. 2003. Abundance and composition of plant species along grazing gradients in Australian grasslands. Journal of Applied Ecology. 40(6): 1008-1024.
22. Magliano, P. N.; Breshears, D. D.; Murray, F.; Niborski, M. J.; Nosetto, M. D.; Zou, C. B.; Jobbágy, E. G. 2023. South American Dry Chaco rangelands: Positive effects of cattle trampling and transit on ecohydrological functioning. Ecological Applications. 33(3): e2800.
23. Merdas, S.; Kouba, Y.; Mostephaoui, T.; Farhi, Y.; Chenchouni, H. 2021. Livestock grazing-induced large-scale biotic homogenization in arid Mediterranean steppe rangelands. Land Degradation & Development. 32(17): 5099-5107.
24. Monger, C.; Bestelmeyer, T. 2006. The soil-geomorphic template and biotic change in arid and semi-arid ecosystems. Journal of Arid Environments. 65: 207-218.
25. Oliva, G.; Paredes, P.; Ferrante, D.; Cepeda, C.; Rabinovich, J. 2019. Remotely sensed primary productivity shows that domestic and native herbivores combined are overgrazing Patagonia. Journal of Applied Ecology. 56(7): 1575-1584.
26. Oñatibia, G. R. 2021. Grazing management and provision of ecosystem services in Patagonian arid rangelands. In Ecosystem Services in Patagonia: A Multi-Criteria Approach for an Integrated Assessment. Springer International Publishing. p. 47-74.
27. Parker, K. C.; Bendix, J. 1996. Landscape-Scale geomorphic Influences on vegetation patterns in four environments. Physical Geography. 17(2): 113-141.
28. Passera, C. B.; Dalmasso, A. D.; Borsetto, O. 1983. Método de “point quadrat modificado”. Taller de arbustos forrajeros para zonas áridas y semiáridas. 71-79.
29. Passera C. B.; Borseto O. 1986. Determinación del “Índice de Calidad Específico”. Actas del Taller de Arbustos Forrajeros. Grupo Regional FAO-IADIZA, Mendoza.
30. QGIS, Equipo de desarrollo. 2020. Sistema de información geográfica de código abierto. Fundación Fuente Geoespacial. Versión 2.18.20. https://qgis.org/es/site
31. Quiroga, R. E.; Blanco, L. J.; Namur, P. R. 2018. Defoliation intensity and effects of simulated grazing strategies in three C4 grasslands. Range Ecology and Management. 71(1): 58-66.
32. Sasaki, T.; Okayasu, T.; Jamsran, U.; Takeuchi, K. 2008. Threshold changes in vegetation along a grazing gradient in Mongolian rangelands. Journal of Ecology. 96(1): 145-154.
33. Scaglia, J. A.; Flores, D. G.; Martinelli, M. 2021. Productividad de los pastizales naturales en diferentes unidades geomorfológicas de las Sierras Pampeanas de Argentina. Ecosistemas. 30(2): 2104-2104.
34. Skarpe, C. 2000. Desertification, no-change or alternative states: Can we trust simple models on livestock impact in dry rangelands? Applied Vegetation Science. 3(2): 261-268.
35. Szymañski, C.; Villagra, P. E.; Aschero, V.; & Alvarez, J. A. 2022. Interactive effects of chronic anthropogenic disturbances on Prosopis forest structure in Monte Central, Argentina. Southern Ecology. 32(1): 108-121. https://doi.org/10.25260/EA.22.32.1.0.1800

36. Vignoni, A. P.; Peralta, I. E.; Abraham, E. M. 2023. Fragmented areas due to agricultural activity: native vegetation dynamics at crop interface (Montecaseros, Mendoza, Argentina). Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 46-60. DOI: https://doi.org/10.48162/rev.39.108
37. Weber, K. T.; Horst, S. 2011. Desertification and livestock grazing: the role of sedentarization, mobility and rest. Pastoralism: Research. Policy and Practice. 1(1): 1-11.
38. Westoby, M.; Walker, B.; Noy-Meir, I. 1989. Opportunistic management of grasslands that are not in equilibrium. Range Ecology and Management/Journal of Range Management Archives. 42(4): 266-274.

Acknowledgements

We thank the anonymous reviewers and English Professor Jimena Olivares for their comments on this article.

This research is supported by INTA - EEA Pocito and CONICET - CCT San Juan. Argentina.

Landform heterogeneity drives multi-stemmed *Neltuma flexuosa* growth dynamics. Implication for the Central Monte Desert forest management

La heterogeneidad de paisaje modula la dinámica en el crecimiento de *Neltuma flexuosa*. Implicancias para el manejo forestal de los bosques del Desierto del Monte Central

Sergio Piraino [1, 2*], Fidel Alejandro Roig [1, 2, 3]

Originales: *Recepción:* 18/09/2023 - *Aceptación:* 14/04/2024

Abstract

Drylands represent the main earth biome, providing ecosytemic services to a large number of people. Along these environments, woodlands are often dominated by multi-stemmed trees, which are exploited by local inhabitants to obtain forest products for their livelihood. In central-west Argentina, *Neltuma flexuosa* (algarrobo) woodlands are distributed across different landform units, varying in topographical and soil characteristics. This research aimed to reconstruct stem-growth time until harvestable diameter was achieved, and biological rotation age according to topo-edaphic variability in three algarrobo forests using dendrochronological methods. Results indicated that landform heterogeneity modulated species radial growth, influencing stem increments and cutting cycle period. In this sense, a decreasing trend in tree productivity emerged along a loamy-to-sandy textured soil gradient. These findings provide useful novel information for *N. flexuosa* forest management, suggesting the need to account for spatial landform/soil heterogeneity when examining desert forest dynamics.

Keywords

arid woodlands • forest management • growth form • tree-rings

1 Laboratorio de Dendrocronología e Historia Ambiental (IANIGLA CONICET). Av. Dr. Adrian Ruiz Leal. Mendoza. M5500. Argentina. * spiraino@fca.uncu.edu.ar

2 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Cátedra de Dasonomía. Almirante Brown 500 (M5528AHB). Chacras de Coria. Mendoza. Argentina.

3 Universidad Mayor. Facultad de Ciencias. Hémera Centro de Observación de la Tierra. Camino La Pirámide 5750. Huechuraba. Santiago. Chile.

Resumen

Las tierras secas representan el principal bioma terrestre, proveyendo numerosos servicios ecosistémicos a un ingente número de personas. En estos ambientes, los bosques son a menudo dominados por árboles de porte multi-fustal, explotados por los habitantes locales para obtener productos forestales útiles para su sustento. En los territorios del centro-oeste de Argentina, los bosques de *Neltuma flexuosa* (algarrobo) están distribuidos en diferentes unidades de paisaje, los que presentan variaciones en las características topográficas y edáficas. En este estudio reconstruimos, a través de métodos dendrocronológicos, el crecimiento, el intervalo para obtener productos maderables, y el turno biológico de corta en función de la variabilidad topo-edáfica en tres bosques diferentes de algarrobo. Los resultados indican que la heterogeneidad del paisaje modula el crecimiento radial de la especie, influenciando el incremento diamétrico y el turno de corta. En este sentido, se evidencia una tendencia decreciente en valores de productividad según un gradiente edáfico desde textura limosa a arenosa. Estos hallazgos proveen información novedosa y de utilidad para los planes de manejo forestal de los bosques de *N. flexuosa*, indicando la necesidad por considerar la heterogeneidad espacial establecidas por las unidades de paisaje/suelos en estudios relativos a la dinámica forestal de los bosques de tierras secas.

Palabras claves

bosques de tierras secas • manejo forestal • forma de crecimiento • ancho de anillo

Introduction

Worldwide, drylands represent the largest biome on Earth, covering more than 40% of land surface (7, 29). These environments, characterized by precipitation/evapotranspiration ratio below 0.2, include 20% of plant diversity hotspots, play a fundamental role in various biogeochemical cycles, and contribute to approximately 40% of global net primary productivity (11, 16, 29).

Drylands host more than two billion people (29). Along these biomes, forests cover approximately 1.400 Mha, providing several ecosystemic services like water and soil regulation, food, biochemical, and raw material provision, as well as cultural services (7, 24). Physiognomically, many desert forests are dominated by tree species capable of regeneration through development of new tissue following disturbance, a physiological trait known as resprouting (8, 30). This results in vegetation with coexisting one- and multi-stemmed trees, which differ in growth, productivity, reproduction, and survival ability (30).

Dryland forests have suffered various types of natural and anthropogenic disturbances, such as, fire, insect outbreak, and drought, in addition to high deforestation rates due to agricultural and livestock expansion (6, 20). In this sense, it is estimated that, globally, about 220,000 km^2 of tree-covered drylands were converted into other land cover types between 1992 and 2015 (29). This suggests the need to sustainably exploit and manage these natural resources, particularly in the actual climate change scenario (16).

The Central Monte Desert (hereafter, CMD) is a dryland biome located in central-west Argentina (2). Along these districts, the algarrobo dulce, *Neltuma flexuosa* (DC.) C.E. Hughes & G.P. Lewis (formerly designated as *Prosopis flexuosa* DC), is a dominant tree species growing on a variety of landform units (20). These woodlands were severely exploited during the first half of the 20th century, mainly for domestic wood demand, railway construction and, afterwards, vineyard expansion, with a resulted extraction of approximately 1 million tons of wood (1, 28).

Nowadays, the *N. flexuosa* CMD forests are mostly managed by the local Huarpe native community, through forestry practices that consider the typical low tree growth rates of these woodlands (28). As a result, logging and thinning are extremely rare activities in this area, whereas other silvicultural approaches, such as pruning and deadwood removal, represent valuable forest management alternatives (4, 27). Pruning plays a prominent role due to the high presence of multi-stemmed trees in CMD forests, as well as their higher productivity with respect to one-stemmed individuals (3, 4).

Previous analyses examined productivity in one- and multi-stemmed *N. flexuosa* trees, providing valuable information regarding the CMD algarrobo forest management, but solely at local scale (4). As previously mentioned, the CMD *N. flexuosa* woodlands grow on a mosaic of landform units, expression of soil variability and topographic characteristics (21). This environmental heterogeneity is reflected in differences of tree-ring development, as well as its relation with precipitation regime and disturbance (21, 22). It could be hypothesized, therefore, that the CMD landform variability would represent a factor modulating multi-stemmed *N. flexuosa* tree productivity, as well as stem-cutting cycle periods. Studying how topo-edaphic characteristics influence multi-stemmed algarrobo growth dynamics can enhance CMD silvicultural management by identifying landforms that can sustain intense forest exploitation without ecological imbalance. For this reason, this research analyzed stem growth rates of several multi-stemmed *N. flexuosa* trees distributed along three different landform units, aiming to reconstruct diametric cumulative increment, time (expressed in years) until forest products can be obtained, and biological rotation age according to variations in topography and soil characteristics of the CMD algarrobo forest stands.

Materials and methods

In this study, we examined three sites within the CMD located in the Province of Mendoza, Argentina, representing distinct landform units (figure 1). Along these districts, the algarrobo woodlands grow under semi-arid climatic conditions, with mean annual precipitation of 155 mm, and large variability in temperature at both daily and seasonal timescales (2). Vegetation consists of the typical CMD plant association, with *N. flexousa* dominating the tree layer, occasionally accompanied by *Geoffroea decorticans*, along with shrub presence of *Larrea divaricata* Cav., (Hook. And Arn.), *Atamisquea emarginata* Miers ex Hook. & Arn and *Bulnesia retama* (Hook.) Griseb (28).

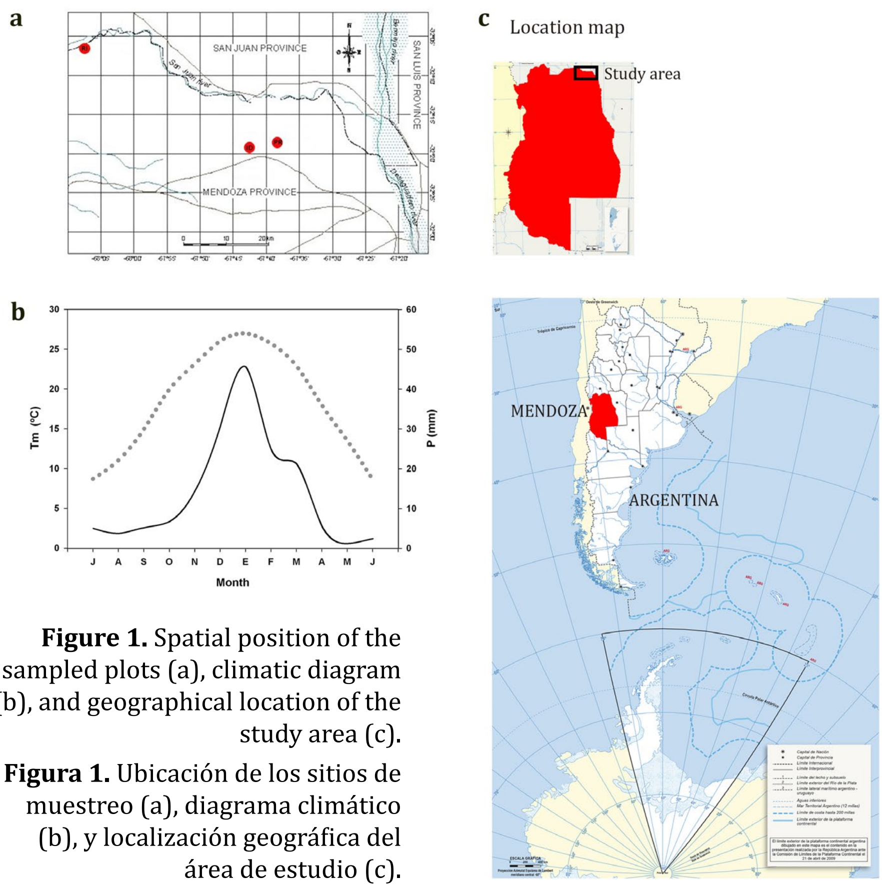


Figure 1. Spatial position of the sampled plots (a), climatic diagram (b), and geographical location of the study area (c).

Figura 1. Ubicación de los sitios de muestreo (a), diagrama climático (b), y localización geográfica del área de estudio (c).

Monthly total precipitation (continuous line) and monthly air temperature (dashed line) data belong to the Encón climatic station (32°15' S, 67°47' W), covering the 1971-1987 period. RI, PR, and ID represent River, Paleo-river, and Inter-dune valley units, respectively.

Los datos de precipitación media mensual (línea continua) y temperatura promedio mensual (línea puntuada) pertenecen a la estación meteorológica de Encón (32°15' S, 67°47' W), para el período 1971-1987. RI, PR, e ID representan respectivamente las unidades Riparia, Paleo-riparia, y de Valle inter-medanoso.

Sites were selected based on prior geomorphological classification (23). In this study, River, Paleo-river, and Inter-dune valley landform units were examined, differing in topographic characteristics and edaphic features (table 1). The selected algarrobo woodlands were in relative proximity (within 50 km), allowing us to assume a common climatic influence upon the species stem growth. Soil types varied among the examined stands, with sandy soils in the River and Interdune valley units and loamy soils in the Paleo-river site (table 1). On the other hand, soil permeability differed among the selected woodlands, showing high to moderate and moderate-hydrophobic characteristics (table 1).

Table 1. Geographical and environmental characteristics of the sampled sites.
Tabla 1. Características geográficas y ambientales de los sitios de muestreo.

Landform Unit	Altitude (m a. s. l.)	Latitude (°S)	Longitude (°W)	Soil texture	Soil permeability
River	534	32°06'26,6''	68°07'26,4''	Sandy	Moderate-hydrophobic
Paleo-river	497	32°18'51,9''	67°38'18,1''	Loamy	Moderate
Inter-dune valley	511	32°19'17,1''	67°42'17,0''	Sandy	High

m a. s. l.: meters above sea level.
m a. s. l.: metros sobre el nivel del mar.

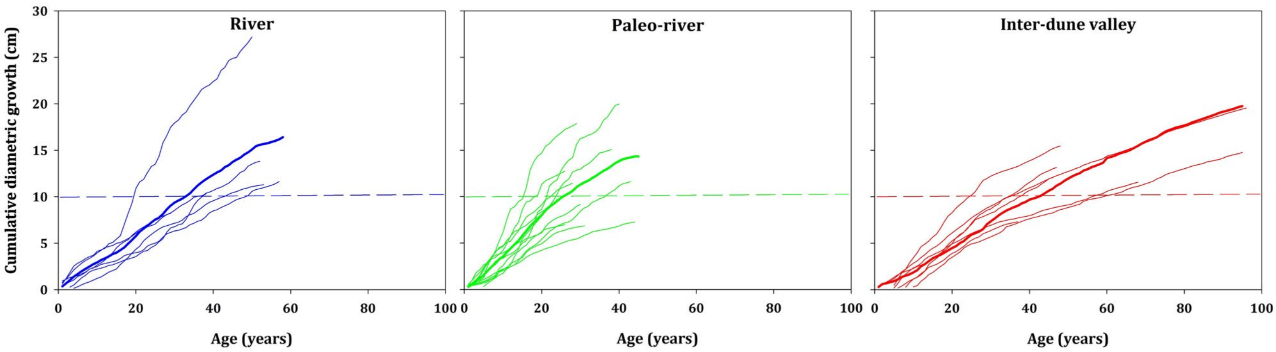
At each site, 1-2 rectangular plots of 1.000m^2 (50m x 20m) were established to examine the algarrobo forest dynamics in terms of tree radial growth relation with rainfall and its response to thinning, results already published (21, 22). The examined stands exhibited variations in structural characteristics, including differences in multi-stemmed tree density, mean basal stem diameter, and tree height, whereas the number of stems per tree was similar among the selected algarrobo forests (table 2).

Table 2. Stand structural characteristics of the sampled sites.
Tabla 2. Características estructurales de los sitios muestreados.

Landform Unit	Density (ms/ha)	Dbase (cm)	H (m)	N° stems/tree
River	60	14,9	4,8	3,8
Paleo-river	110	13,8	5.3	3,2
Inter-dune valley	70	17,2	7,1	3,7

ms: multi-stemmed tree. Dbase: mean multi-stemmed tree basal diameter; H: mean multi-stemmed tree height.
ms: árbol multi-fustal. Dbase: diámetro basal promedio del árbol multi-fustal. H: altura promedio del árbol multi-fustal.

For each multi-stemmed tree, two samples were extracted with a gas-powered drill (TED_262R, Tanaka Kogyo Co. Ltd, Chiba, Japan), at approximately 50 cm above the ground from the stem with the highest basal diameter. Samples were air-drained, mounted on a wooden support, and polished with progressively finer sandpaper to highlight wood anatomy. Rings were dated following the Schulman criteria for the Southern Hemisphere tree species (25). Tree-ring widths were measured from pith to bark with a 0.01 mm resolution Velmex table connected to a computer. The correct dating of these measurements was statistically validated with COFECHA software (13). Then, we estimated individual tree diametric increments by averaging ring measurements from samples belonging to each algarrobo tree and multiplying these values by two. Finally, we calculated the cumulative diametric increment (CDI) as the sum of the current annual increments in diameter for each biological year. This information allowed us to estimate the time, in absolute tree age, required for the stem to reach the minimum diameter corresponding to forest products in CMD algarrobo woodlands, based on available information for the studied area (poles: stem ø= 10 cm) (19). When the core center was not reached, tree age was estimated through a geometric method (10). We used ANOVA with post-hoc Fisher's least significant difference test through the Infostat software (9) to compare tree age data across landform units. Five trees were excluded from this analysis since they did not reach minimum CDI values of 10 cm (figure 2).



Dashed horizontal lines show the minimum stem ø = 10 cm. / Las líneas puntuadas indican el ø mínimo del fuste = 10 cm.

Figure 2. Cumulative diameter increment for the River, Paleo-river, and Inter-dune valley landform units.
Figura 2. Crecimiento diamétrico cumulativo para las unidades de paisaje de Ripario, Paleo-ripario, y valle inter-medanoso.

Biological rotation age of the sampled algarrobo trees was estimated considering raw ring widths converted to basal area increment (BAI). First, tree-ring measurements from the two radii, corresponding to the two samples extracted for each tree, were averaged at tree level to obtain individual ring-width chronologies. Then, the AGE routine included in the DPL software (Dendrochronological Program Library) (14) was used to estimate current (CBAI) and mean (MBAI) basal area, according to the following equation:

$$\mathbf{CBAI_t = \pi * (r_t^2 - r_{t-1}^2)} \text{ and } \mathbf{MBAI_t = \pi * (r_t^2)/t}$$

where:

r_t represents ring width in year *t*. Biological rotation age (cutting cycle period) was determined at the age when MBAI and CBAI intersected (5).

Results

Tree-ring width chronologies were constructed using 36 samples corresponding to 24 *N. flexuosa* multi-stemmed trees. The relatively low number of selected trees at River and Inter-dune valley depended on landowner sampling permission. Multi-stemmed algarrobo site chronologies spanned from 44 (Paleo-river unit) to 96 years (Inter-dune valley unit). Mean annual radial growth rates oscillated between 1.27 (Inter-dune valley unit) and 1.77 mm/yr (Paleo-river unit). Mean correlation values (MC) between individual tree-ring chronologies ranked from 0.446 (Inter-dune valley unit) to 0.490 (Paleo-river unit), with all values being significant at $p < 0.05$ (table 3, page 31).

CDI analysis revealed that multi-stemmed trees achieved, on average, a minimum diameter of 10 cm at different ages: 37 years for River unit, 24 years for Paleo-river environment, and 44 years for Inter-dune valley landform (figure 2).

Table 3. Characteristics of the tree-ring chronologies.

Tabla 3. Características de las cronologías de ancho de anillo.

Landform Unit	N	Period	RW (mm)	MC
River	6 (9)	1954-2011	1.38	0.463
Paleo-river	11 (15)	1967-2010	1.77	0.490
Inter-dune valley	7 (12)	1916-2011	1.27	0.446

N = Number of sampled trees per site (in parenthesis: total number of dendrochronological samples); Period = time range of the sampled cores; RW = mean ring-width value; MC = mean correlation between individual tree-ring series at each stand.

N = número de los árboles muestreados (entre paréntesis: número total de las muestras dendrocronológicas); Period = intervalo temporal de las muestras; RW = valor de ancho de anillo promedio; MC = correlación promedio entre las series dendrocronológicas individuales para cada sitio.

Statistically significant differences emerged regarding tree age corresponding to CDI ø= 10 cm among Paleo-river units on one side, and River and Inter-dune valley landforms on the other (one-way ANOVA, F = 5.40, $p = 0.0161$, $df = 2$, n = 19; figure 3).

Each box shows the values within one interquartile distance (ID 25% above and below the median). The median is shown as a black horizontal bar in the boxes. Whiskers represent values reaching 1.5 times the IDs. Different letters indicate significant differences at $p < 0.05$.

Cada caja muestra los valores comprendidos en la distancia de un intercuartil (ID 25% por encima y por debajo del promedio). El promedio se muestra como línea negra. Los bigotes corresponden a un valor de 1,5 veces la distancia de un ID y se muestran como líneas negras. Letras diferentes indican diferencias significativas por $p < 0,05$.

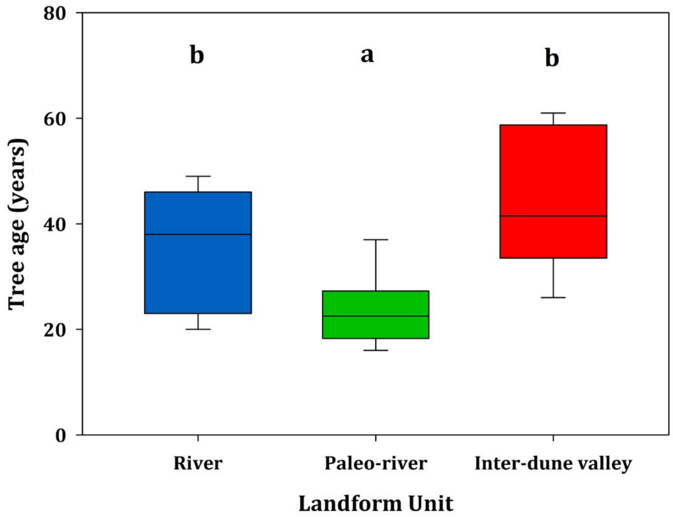
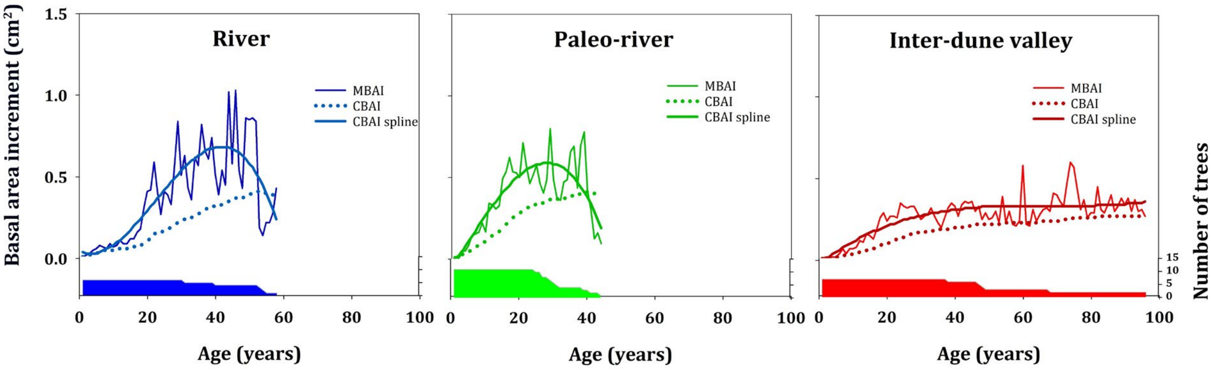


Figure 3. Box and whiskers plot for tree age corresponding to the minimum harvestable diameter of 10 cm across landform units.

Figura 3. Gráficos de caja-bigote para los valores del diámetro mínimo maderable de 10 cm según la unidad de paisaje.

Landform heterogeneity was reflected in biological rotation age among the selected units. In this sense, the cutting cycle period occurred at ages 53 and 41 years at River and Paleo-river units, corresponding to stem diameters of 19 and 18 cm, respectively. In contrast, at the Inter-dune valley landform, the culmination age exceeded 96 years (stem ø = 24.5 cm), corresponding to the age of the two oldest sampled trees (figure 4, page 32).



Coloured areas show the number of trees used for estimating MBAI and CBAI.
Las áreas coloreadas indican el número de árboles utilizados en el cálculo de MBAI y CBAI.

Figure 4. Mean (MBAI) and current (CBAI) annual basal area increments in relation to tree ages across landform units.
Figura 4. Incrementos anuales en área basal promedio (MBAI) y corriente (CBAI) en relación a la edad del árbol según la unidad de paisaje.

Discussion

In this study, we employed dendrochronological methods to reconstruct growth dynamics of multi-stemmed *N. flexuosa* across three distinct landform units within the CMD. This research provides pioneering evidence of how topographical and edaphic factors influence growth rates of multi-stemmed algarrobo trees in the studied area. Our investigation delivered novel insights concerning the most important forest resource that sustains local communities residing in arid central-west Argentina. The low number of examined multi-stemmed *N. flexuosa* individuals at two sites is not considered an analytic limitation, aimed to explore for the first time tree growth form-dependent dynamics at regional scale. Future studies increasing site repetitions as well total sampled tree number could validate our findings.

Our analyses demonstrated that landform variations were mirrored by growth rates of multi-stemmed algarrobo trees, evidenced by differences in the time required to attain harvestable stem diameters, and the length of the cutting cycle period. Previous dendrochronological assessments suggested that soil attributes and environmental heterogeneity play pivotal roles in shaping moisture availability in the CMD districts, thereby influencing growth dynamics of algarrobo woodlands (21). In this sense, it is worth mentioning that *N. flexuosa* exhibits characteristics of a facultative phreatophyte species, with distinctive root architecture that enables algarrobo trees to access deep phreatic water while also exploring shallow soil horizons (12). This trait allows *N. flexuosa* trees to use both precipitation and groundwater for tree growth (15). Across the woodlands examined in our study, the phreatic water table consistently lies at similar depths, approximately between 4.5 and 6 meters (26), suggesting that factors beyond water table accessibility, likely soil characteristics and impact on water retention of superficial horizons, probably contributed to the observed variations in tree growth. In arid ecosystems like the CMD, sandy soils tend to facilitate deep water infiltration, whereas loamy edaphic layers promote water retention in superficial horizons, resulting in enhanced stem growth (17, 18, 21). Moreover, soil permeability directly influences water storage capacity, with moderately permeable soils exhibiting better precipitation retention compared to those with higher permeability (18, 21). Both of these edaphic attributes may collectively account for our research outcomes.

Previous analyses have indicated that the biological rotation age of *N. flexuosa* is growth-form dependent, with multi-stemmed trees reaching the cutting cycle period at younger age than their one-stemmed counterparts (4). According to Alvarez *et al.* (2011), multi-stemmed algarrobo trees could be harvested at approximately 80 years of age.

However, our study underscores that landform clearly modulated the cutting cycle period. Our findings revealed different values for biological rotation age across the examined stand. Regarding the discrepancy observed for similar environments, that is, between the Inter-dune valley stand analyzed in this research and the woodland examined by Alvarez *et al.* (2011), belonging to the same landform unit, it could be hypothesized that variations in topographical features, such as the presence (4) or absence (our study) of a complete dune system surrounding the Inter-dune valley site, may affect runoff processes and hence water availability. This landform variability could potentially account for this particular divergent result, although further investigations are warranted to explore the specific topographic influence at a smaller spatial scale within the Inter-dune valley unit, to address this difference more comprehensively.

At local scale, our results indicated that wood extraction from multi-stemmed *N. flexuosa* in CMD districts should consider landform heterogeneity. Specifically, while multi-stemmed algarrobo trees in loamy Paleo-river soil and, to a lesser extent, River units could be managed for pole production, pruning should be avoided in Inter-dune valley *N. flexuosa* forest stands. This recommendation is grounded in the exceptionally extended time required to reach biological rotation age in this landform, which is approximately 100 years. Additionally, the slow radial growth rates in the Inter dune valley environment translated to an extended time window for obtaining forest products used by local inhabitants in the studied area.

Conclusions

This research sheds crucial light on a strategically vital forest resource for the communities residing in the CMD area. These findings should be integrated with the development of future forest management strategies for the semi-arid *N. flexuosa* woodlands located in the central-western region of Argentina. On a broader scale, our study underscores the paramount importance of considering landform heterogeneity in the management of desert resprouting woody vegetation.

References

1. Abraham, E. M.; Prieto, M. R. 1999. Vitivinicultura y desertificación en Mendoza. En: García Martínez, B. (Ed.), Estudios de historia y ambiente en América: Argentina, Bolivia, México, Paraguay. IPGH-Colegio de México, México. 109-135.
2. Abraham, E.; del Valle, H. F.; Roig, F.; Torres, L.; Ares, J. O.; Coronato, F.; Godagnone, R. 2009. Overview of the geography of the Monte Desert biome (Argentina). Journal of Arid Environments. 73(2): 144-153.
3. Alvarez, J. A.; Villagra, P. E.; Cony, M. A.; Cesca, E.; Boninsegna, J. A. 2006. Estructura y estado de conservación de los bosques de *Prosopis flexuosa* DC (Fabaceae, subfamilia: Mimosoideae) en el noreste de Mendoza (Argentina). Revista Chilena de Historia Natural. 79(1): 75-87.
4. Alvarez, J. A.; Villagra, P. E.; Villalba, R.; Cony, M. A.; Alberto, M. 2011b. Wood productivity of *Prosopis flexuosa* DC woodlands in the central Monte: Influence of population structure and treegrowth habit. Journal of Arid Environments. 75(1): 7-13.
5. Assman, E. 1970. The principles of forest yield study. Section B: Tree growth and form, Pergamon Press. New York. 51-53.
6. Baldassini, P.; Bagnato, C. E.; Paruelo, J. M. 2020. How may deforestation rates and political instruments affect land use patterns and Carbon emissions in the semi-arid Chaco, Argentina?. Land Use Policy. 99: 104985.
7. Bastin, J. F.; Berrahmouni, N.; Grainger, A.; Maniatis, D.; Mollicone, D.; Moore, R., Patriarca, C.; Picard, N.; Sparrow, B.; Abraham, E. M.; Aloui, K.; Atesoglu, A.; Attore, F.; Bassüllü, C.; Bey, A.; Garzuglia, M.; García-Montero, L. G.; Groot, N.; Guerin, G.; Laestadius, L.; Lowe, A.; Mamane, B.; Marchi, G.; Patterson, P.; Rezende, M.; Ricci, S.; Salcedo, I.; Sanchez-Diaz Paus, A.; Stolle, F.; Surappaeva, V.; Castro, R. 2017. The extent of forest in dryland biomes. Science. 356(6338): 635-638.
8. Bond, W. J.; Midgley, J. J. 2001. Ecology of sprouting in woody plants: the persistence niche. Trends in ecology & evolution. 16(1): 45-51.
9. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; González, L.; Tablada, M.; Robledo, Y. C. 2011. InfoStat versión 2011. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www. infostat.com.ar. 8: 195-199.
10. Duncan, R. P. 1989. An evaluation of errors in tree age estimates based on increment cores in

kahikatea (*Dacrycarpus dacrydioides*). New Zealand Natural Sci. 16: 31-37.
11. FAO (Food and Agriculture Organization). 1977. Carte mondiale de la désertification a l'échelle de 1:25,000,000, Conférence des Nations Unies sur la désertification, 29 août–9 septembre, A/CONF. 74/2.
12. Guevara, A.; Giordano, C. V.; Aranibar, J.; Quiroga, M.; Villagra, P. E. 2010. Phenotypic plasticity of the coarse root system of *Prosopis flexuosa*, a phreatophyte tree, in the Monte Desert (Argentina). Plant and soil. 330(1-2): 447-464.
13. Holmes R. L. 1983. Computer-assisted quality control in tree-ring dating and measurement. Tree-Ring Bulletin. 43: 69-78.
14. Holmes, R. L. 1999. Dendrochronological Program Library (DPL). User's Manual, Laboratory of Tree-Ring Research, University of Arizona. Tucson. Arizona. USA.
15. Jobbágy, E. G.; Nosetto, M. D.; Villagra, P. E.; Jackson, R. B. 2011. Water subsidies from mountains to deserts: their role in sustaining groundwater-fed oases in a sandy landscape. Ecological Applications. 21(3): 678-694.
16. Maestre, F. T.; Benito, B. M.; Berdugo, M.; Concostrina-Zubiri, L.; Delgado-Baquerizo, M.; Eldridge, D. J.; Guirado, E.; Gross, N.; Kéfi, S.; Le BagoussePinguet, Y.; Ochoa-Hueso, R.; Soliveres, S. 2021. Biogeography of global drylands. New Phytologist. 231(2): 540-558.
17. Massa, A.; Quagliariello, G.; Martinengo, N.; Calderón, A.; Pérez, S. 2023. Growth and slenderness index in sweet algarrobo, *Neltuma flexuosa*, according to the vermicompost percentage in the substrate and seed origin. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 12-19. DOI: https://doi.org/10.48162/rev.39.105
18. Noy-Meir, I. 1973. Desert ecosystems: environment and producers. Annual review of ecology and systematics. 4: 25-51.
19. Ongay Ugarteche, O.; Marambio Fermani. S.; Corominas Day, M.; Lagos Slinik, S. Acordinaro N. 2011. Manual de Bosques Nativos. Un aporte a la Conservación desde la Educación Ambiental. Dirección de Recursos Naturales Renovables Secretaría de Medio Ambiente Gobierno de Mendoza. 121 p.
20. Petrie, M. D.; Bradford, J. B.; Hubbard, R. M.; Lauenroth, W. K.; Andrews, C. M.; Schlaepfer, D. R. 2017. Climate change may restrict dryland forest regeneration in the 21^{st} century. Ecology. 98(6): 1548-1559.
21. Piraino, S.; Abraham, E. M.; Diblasi, A.; Roig Juñent, F. A. 2015. Geomorphological-related heterogeneity as reflected in tree growth and its relationships with climate of Monte Desert *Prosopis flexuosa* DC woodlands. Trees. 29: 903-916.
22. Piraino, S.; Abraham, E. M.; Hadad, M. A.; Patón, D.; Roig-Juñent, F. A. 2017. Anthropogenic disturbance impact on the stem growth of *Prosopis flexuosa* DC forests in the Monte desert of Argentina: A dendroecological approach. Dendrochronologia. 42: 63-72.
23. Rubio, M. C.; Soria, D.; Salomón, M. A.; Abraham, E. 2009. Delimitación de unidades geomorfológicas mediante la aplicación de técnicas de procesamiento digital de imágenes y SIG. Área no irrigada del departamento de Lavalle, Mendoza. Proyección. 2: 1-33.
24. Schild, J. E.; Vermaat, J. E.; van Bodegom, P. M. 2018. Differential effects of valuation method and ecosystem type on the monetary valuation of dryland ecosystem services: A quantitative analysis. Journal of arid environments. 159: 11-21.
25. Schulman, E. 1956. Dendroclimatic changes in semiarid America. University of Arizona Press, Tucson.
26. Soria, D.; Rubio, C.; Abraham, E. M. 2011. Sitio piloto en la Región Centro Oeste. En: "Evaluación de la desertificación en Argentina. Resultados del proyecto LADA/FAO". FAO, PAN, UNEP, GEF, LADA y SAyDS. Gráfica Latina. Buenos Aires, Argentina. 205-246.
27. Vázquez, D. P.; Alvarez, J. A.; Debandi, G.; Aranibar, J. N.; Villagra, P. E. 2011. Ecological consequences of dead wood extraction in an arid ecosystem. Basic and Applied Ecology. 12(8): 722-732.
28. Villagra, P. E.; Defossé, G. E.; Del Valle, H. F.; Tabeni, S.; Rostagno, M.; Cesca, E.; Abraham, E. 2009. Land use and disturbance effects on the dynamics of natural ecosystems of the Monte Desert: Implications for their management. Journal of Arid Environments. 73(2): 202-211.
29. Wang, L.; Jiao, W.; MacBean, N.; Rulli, M. C.; Manzoni, S.; Vico, G.; D'Odorico, P. 2022. Dryland productivity under a changing climate. Nature Climate Change. 12(11): 981-994.
30. Ware, I. M.; Ostertag, R.; Cordell, S.; Giardina, C. P.; Sack, L., Medeiros, C. D.; Inman, F.; Litton, C. M.; Giambelluca, T.; John, G. P.; Scoffoni, C. 2022. Multi-stemmed habit in trees contributes climate resilience in tropical dry forest. Sustainability. 14(11). 6779.

Acknowledgments

The authors warmly thank the Aguero and Cordoba families for allowing sampling in their respective areas. Special thanks are due to Hugo Debandi and Alberto Ripalta for field assistance. We thank the Dirección de Recursos Naturales Renovables of Mendoza Province for allowing sampling.

Rural abandonment and its drivers in an irrigated area of Mendoza (Argentina)

Abandono rural y sus causas en un área irrigada de Mendoza (Argentina)

Bárbara Guida-Johnson [1, 2*], Ana Paz Vignoni [3, 4], Gabriela M. Migale [5],
María Agustina Aranda [2], Andrea Magnano [3]†

Originales: *Recepción:* 26/09/2023 - *Aceptación:* 09/05/2024

Abstract

Rural abandonment is a global phenomenon promoted by biophysical, socio-economic, and socio-productive causes, leading to the disappearance of traditional agricultural practices and serious impacts on food security and local livelihoods. This phenomenon is more complex in drylands since the lost of productive land is unlikely to be recovered due to the limited availability of water resources. This study aimed to identify abandoned agricultural lands in a sector located east of the northern oasis of Mendoza (Argentina) and determine the main driving forces leading this process. The interdisciplinary perspective employed included the Normalized Difference Vegetation Index (NDVI) difference technique implemented on Landsat images, the boosted regression trees analysis of spatially explicit drivers, and a digital survey providing perception assessments from local producers and their technical advisors. Abandoned agricultural land has increased by 92% between 2002 and 2020, being accessibility, crop type, vulnerable living conditions of the local population, availability of irrigation water and labor, and the lack of profitability, the main drivers identified by both sources of information (spatial model and social perception). The proposed approach contributes to monitore productive resources and land-use planning with a holistic and long-term vision.

Keywords

agricultural land abandonment • NDVI difference technique • spatial modeling • social actors' perception

1 Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA), Universidad Nacional de Cuyo, Gobierno de Mendoza, CONICET. Av. Ruiz Leal s/n (M5500). * bguidaj@mendoza-conicet.gob.ar

2 Universidad Nacional de Cuyo. Facultad de Ciencias Exactas y Naturales. Padre Jorge Contreras 1300 (M5502JMA) Mendoza. Argentina.

3 Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA), Universidad Nacional de Cuyo, Gobierno de Mendoza, CONICET. Av. Ruiz Leal s/n (M5500).

4 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Almirante Brown 500 (M5528AHB) Mendoza. Argentina.

5 Universidad de Buenos Aires. Facultad de Filosofía y Letras. Puan 480 (C1406CQJ). Ciudad Autónoma de Buenos Aires. Argentina.

Resumen

El abandono rural es un fenómeno global promovido por causas biofísicas, socioeconómicas y socioproductivas, que conduce a la desaparición de prácticas agrícolas tradicionales y tiene graves impactos en la seguridad alimentaria y los medios de vida locales. Este fenómeno es más complejo en las tierras secas, ya que es poco probable que se recuperen las tierras productivas perdidas debido a la disponibilidad limitada de recursos hídricos. Los objetivos de este estudio son identificar tierras agrícolas abandonadas en un sector del este del oasis norte de Mendoza (Argentina) y determinar las principales causas que conducen este proceso. Se empleó una perspectiva interdisciplinaria, que incluye la implementación de la técnica de diferencia del Índice de Vegetación de Diferencia Normalizada (NDVI) a partir de imágenes Landsat, el análisis de *boosted regression trees* para evaluar causas espacialmente explícitas y la evaluación de la percepción de los productores locales y sus asesores técnicos mediante una encuesta digital. Las tierras agrícolas abandonadas aumentaron un 92% entre 2002 y 2020 y los principales factores identificados por ambas fuentes de información (modelo espacial y percepción social) fueron la accesibilidad, el tipo de cultivo, las condiciones de vida vulnerables de la población local, la disponibilidad de agua de riego, la disponibilidad de mano de obra y la falta de rentabilidad. El enfoque propuesto busca contribuir al monitoreo de los recursos productivos y a un ordenamiento territorial con una visión holística y de largo plazo.

Palabras clave

abandono de tierra agrícola • técnica de diferencia del NDVI • modelado espacial • percepción de los actores sociales

Introduction

Rural abandonment is a global phenomenon detected in diverse regions, such as Europe, the United States, Asia, and Latin America (40). In general terms, rural abandonment is mainly promoted by three types of causes: biophysical (including factors such as elevation and slope, soil, or climate), socio-economic (including market incentives, migration processes, inefficient technology, land tenure, accessibility or characteristics of the producers) and, thirdly, socio-productive (associated with inadequate agricultural management leading to land degradation, overexploitation or loss of productivity) (35). Abandonment of productive land generally occurs in marginal agricultural areas and is associated with low levels of profitability (3, 40). This affects crop productivity even more and undermines investment, technological advancement, and the achievement of acceptable economic returns deepening the abandonment processes (37). Some authors see this change in agricultural land use as an opportunity for the restoration of ecosystem services and the recovery of biodiversity (19, 40), depending on the level of degradation. However, the social consequences of rural abandonment are extremely negative and include the disappearance of traditional practices, seriously impacting food security and local livelihoods (9, 12).

According to the UNCCD (2014), 52% of the world's agricultural land is moderately or severely affected by land degradation. Based on current consumption trends, it is estimated that agricultural production will need to increase by 70% globally, and 100% in developing countries, to meet population demands in 2050. Paradoxically, in Latin America, 50% of agricultural land could be affected by desertification by the same year (42). In this context and especially in drylands, inadequate management of productive resources puts the producers' subsistence and livelihoods at risk. Drylands account for 47% of the earth's land surface, covering hyper-arid, arid, semi-arid, and dry sub-humid climate regions where 2.6 billion people live (23). In Argentina, 63% of the national territory constitutes drylands (43). In these regions, where different irrigation systems transform arid ecosystems into cultivable irrigated areas known as "oases", water limits agricultural production. This limitation further complicates the phenomenon of rural abandonment. In arid to semi-arid Mendoza (Argentina) with 250 mm average rainfall, irrigation depends on water from snowmelt in the Andes Mountains, which feeds surface and underground water sources.

Thus, after years of gaining desert land through costly installations of irrigation systems, productive land is lost with low replacement prospects.

In this context, information systems have become valuable tools for managing datasets and identifying critical areas affected by land degradation processes (37). In particular, rural abandonment involves complex dynamics that, as such, must be addressed from an interdisciplinary perspective, including biophysical and socio-economic aspects. Therefore, the construction of spatial models in geographic information systems (GIS) and their statistical analysis makes it possible to associate local data on the occurrence of rural abandonment with variables that describe the biophysical environment and anthropogenic uses. Moreover, each variable has a relative assigned weight reflecting its influence on the process. This approach has allowed several case studies (12, 30, 33, 40). Given this multicausal problem, producer perspectives are valuable assets for understanding perceived constraints, threats, and opportunities (10, 13). Some studies have explored their perceptions of abandonment and plausible causes (4, 24, 31), while others have integrated quantitative data derived from spatial analysis with qualitative data derived from social studies to analyze landscape changes (14, 16, 32). Sustainability demands combining knowledge from different sources to achieve deeper understanding of any given problem.

Rural abandonment has been identified as an environmental problem in the oases of Mendoza but has not yet been thoroughly quantified (1). A previous study identified land-use changes between 2003 and 2019 in a district of the Guaymallén department located in the northern oases (15). Although the authors did identify the category of abandoned land, the employed methodology had limited scalability since it consisted of a visual interpretation of high-definition satellite images available on Google Earth. Both the observer training as well as image availability may hamper analysis extension to the entire oases. Another recent study identified land use and land cover changes between 1986 and 2018 at a larger scale, the Mendoza and Tunuyán River Basins (36). In this case, the methodology enabled an extensive analysis but did not explicitly identify the category of abandoned land. Regarding causes of productive land abandonment, Guida-Johnson *et al.* (2020) investigated the perception of local actors but did not intend to perform spatial modeling. Our study complements previous work and deepens the level of analysis. Rural abandonment is a complex process that can be addressed, quantified, and expressed spatially through an appropriate methodology. In this sense, detecting crop abandonment and investigating possible causes will significantly contribute to understanding rural abandonment dynamics at a local scale. Our objectives were: (1) to identify abandoned land in an irrigated area in the north of Mendoza province between 2002 and 2020 and (2) to determine the main biophysical, socio-economic, and socio-productive drivers considering spatial modeling and social actors perception.

Materials and methods

Study area

The productive activities in Mendoza province (Argentina) are concentrated in three oases: north (irrigated by the Mendoza and lower Tunuyán rivers), center or Valle de Uco (irrigated by the upper Tunuyán river), and south (irrigated by the Diamante and Atuel rivers). In particular, the northern oasis can be divided into two zones with distinctive characteristics: east and center. The study area was the department of General San Martín (figure 1, page 38), good representative of the process of rural abandonment in eastern Mendoza. Located in the Mendoza plain, the department has an area of 1,507 km^2, with its capital city located 43 km from the provincial capital. San Martín is characterized by a great diversity of soils and subsoil water availability, which makes it suitable for crop development. The predominant natural vegetation is xeric, psammophilous, or halophilous shrub steppe, scrubland, and some small relicts of forest (5, 44). Land degradation processes affect the entire area and accelerate the natural phenomena of water and wind erosion (2). According to the latest census (18), the department has almost 140,000 inhabitants and a population density of 92.8 inhabitants/km^2, most of them concentrated in the capital city, and the rest distributed in small or medium-sized towns surrounding the main city.

Approximately 60% of the population over 5 years of age attended some educational establishment (18). Considering population density, health and service infrastructure, and logistics, San Martín is the main department of eastern Mendoza. However, inequalities between urban and rural populations regarding access to social infrastructure (such as drinking water, public transportation, sewage, natural gas, electricity, or waste collection) are profound. Considered one of the main grape-growing areas in the country, the main economic activity is viticulture, followed by olive and fruit production, as well as horticulture. The main economic units are associated industries and family businesses. Historically, urban expansion over rural areas has been carried out in a chaotic or disorderly manner, transforming lands with high agricultural potential and accentuating territorial imbalances.

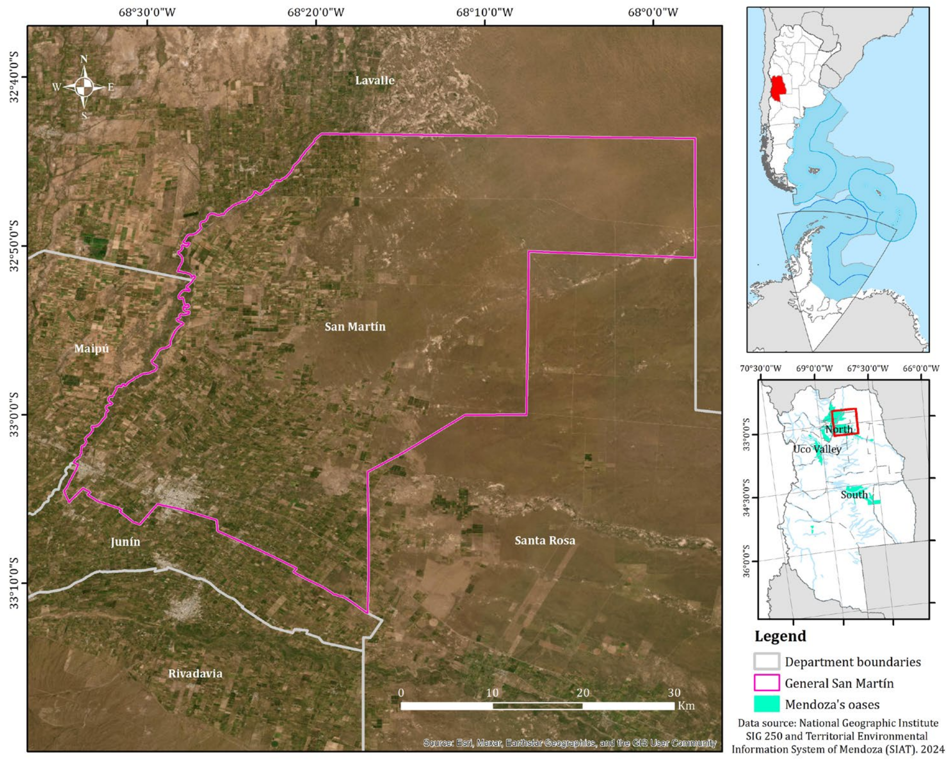


Figure 1. Location of the study area.

Figura 1. Localización del área de estudio.

Detecting rural abandonment

A land-use land-cover (LULC) map was used as a reference and satellite images were analyzed by the Normalized Difference Vegetation Index (NDVI) difference technique to detect changes (22, 27, 41). The production map of Mendoza province constituted our reference. The map, prepared and provided by the Institute for Rural Development [Instituto de Desarrollo Rural, IDR], was created by visual interpretation of high-resolution images available on Google Earth. LULC categories were: abandoned land, non-productive tree cover, fruit trees, vegetables, natural vegetation, olives, pastures, other uses (including swimming pools, and campsites), urban, vines, and vines with olives.

The NDVI difference technique is one algebraic change detection technique, simple and easy to implement and interpret (25). Considering the reference map, the study period was defined between 2002 and 2020. This study used two atmospherically corrected satellite images transformed to surface reflectance: one from the Landsat 5 TM sensor obtained in 2002 and one from Landsat 8 OLI obtained in 2020. A preliminary assessment of monthly NDVI variation associated with different LULC categories allowed image date selection. In

December 2019, 200 field points were surveyed. The largest differences between cultivated and abandoned land were recorded in February when both selected satellite images were obtained. The NDVI was calculated for each scene from the reflectance values in the near-infrared (NIR) and red (R) regions, following Eq. (1).

$$NDVI = \frac{NIR - R}{NIR + R} \quad (1)$$

The NDVI difference was then calculated (ΔNDVI) (Eq. 2). In the resulting image, pixels were divided into no-change pixels with values around zero and change pixels with values sufficiently far from zero (positive and negative) (29).

$$\Delta NDVI = NDVIt2 - NDVIt1 \quad (2)$$

Where NDVIt2 is calculated for the Landsat scene obtained in 2020 and NDVIt1 is calculated for the Landsat scene obtained in 2002. One key issue is threshold definition, *i.e.*, which value change is to be considered significant. In general, thresholds associated with different numbers of standard deviations for NDVI values (σ) are tested, visually choosing the one that best detects changes (27, 29, 41). In this study, four thresholds were tested: 1σ, 1.5σ, 2σ, and the one determined by the Jenks natural breaks classification method.

The changed pixels interpretation was based on comparisons with the reference map considering whether ΔNDVI was positive or negative. Detection of abandoned rural land was performed as detailed in Table 1: no-change pixels preserve their category, whereas change pixels are converted into abandoned land (AL), cultivated land (CL) or not cultivated land (NCL). A rural abandonment map was produced for each ΔNDVI threshold. Original LULC categories from the reference map were regrouped into abandoned land, cultivated land (including fruit trees, vegetables, olives, pastures, vines, and vines with olives) and not cultivated land (non-productive tree cover, natural vegetation, other uses, and urban).

Table 1. Criteria to detect abandoned agricultural land based on NDVI difference technique and a reference map.

Tabla 1. Criterios para detectar tierra agrícola abandonada basándose en la técnica de diferencia del NDVI y un mapa de referencia.

Reference map		**Rural abandonment map**		
		No-change pixels	**Change pixels**	
		ΔNDVI near 0	**Significantly (-) ΔNDVI**	**Significantly (+) ΔNDVI**
Abandoned land	AL	AL	AL	CL
Fruit crops	CL	CL	AL	CL
Vegetable crops				
Olive crops				
Pasture				
Vine crops				
Vine and olive crops				
Natural vegetation	NCL	NCL	NCL	CL
Unproductive tree cover		NCL	NCL	NCL
Other land uses				
Urban				

The ΔNDVI thresholds were assessed by validating the rural abandonment maps from ground truth data. Three campaigns surveying GPS points in the study area were conducted in December 2019, March 2021, and June 2021, collecting 537 sample points. An error matrix was constructed for each map with these data, while descriptive statistics were calculated. Total accuracy represents the proportion of reference points correctly classified. Producer's accuracy indicates the probability of a reference point correctly classified, *i.e.* how properly a particular area can be classified. Finaly, user's accuracy estimates the probability of a point on the map representing the class on the ground (6). A rural abandonment map was selected based on statistics, and the area associated with each category was calculated.

Identifying rural abandonment drivers

Rural abandonment drivers were identified and assessed using two sources of information: a spatial model, and social actors perceptions. A GIS model weighed the relative contribution of spatially explicit driving forces (12, 33). To that end, 2000 points were randomly plotted on the selected rural abandonment map to gather data and run a machine learning algorithm. For each point, the response variable was presence or absence of rural abandonment. Predictor variables were compiled from available geo-referenced secondary data of three types, namely attributes of the biophysical environment, and socio-economic and socio-productive aspects. The former included: (1) elevation obtained from Shuttle Radar Topography Mission (SRTM) data (20); (2) aridity index values, freely available from the Territorial Environmental Information System of Mendoza [Sistema de Información Ambiental Territorial de Mendoza] (38); and (3) frequency of storm damage events recorded during 2002-2017, available at the Directorate of Agriculture and Climatic Contingencies of Mendoza website [Dirección de Agricultura y Contingencias Climáticas de Mendoza] (7). The socio-economic and socio-productive aspects included: (4) the original type of crop obtained from the productive map of Mendoza provided by the Institute for Rural Development; (5) population density, that is the number of inhabitants registered by census unit, (6) the percentage of households with unsatisfied basic needs (UBN), including overcrowded homes, precarious housing, poor sanitary conditions, non schooled children or household head without complete schooling, and (7) percentage of the working-age population who only reached primary education level (*i.e.* those not meeting certain minimum conditions - secondary education – in order to access formal employment), with data obtained from the National Census of Population, Households and Dwellings 2010 [Censo Nacional de Población, Hogares y Viviendas 2010] (17); (8) the Euclidean distance to national and provincial roads available in the Territorial Environmental Information System of Mendoza (39); (9) the Euclidean distance to irrigation network and (10) to groundwater extraction wells and (11) the density of canals and (12) wells, with data provided by the General Department of Irrigation of Mendoza [Departamento General de Irrigación (DGI)]. The values of all the predictor variables were recorded at each random point.

The data were analyzed by boosted regression trees (BRT) (30, 40) through R software (34) using the dismo package version 1.3-5. This approach uses boosting to combine large numbers of relatively simple tree models to optimize predictive performance and identify relevant variables, explaining an observed pattern (11). Model parametrization was performed with tree complexity, which controls the number of fitted interactions; the learning rate, which determines tree contribution to the model; and the bag fraction, which defines the proportion of data selected at each step (11). For this, different combinations of tree complexity (1 to 9), learning rate (0.01, 0.005, 0.001, and 0.0005), and bag fraction (0.5 and 0.75) were tested (21, 28). Model selecting criteria intended to maximize the explained deviance, *i.e.* variability explained by the model, and minimized the difference between the training data AUC score and the cross-validated AUC score, to reduce overfitting (8, 11). Variables with relative influence under the expected by chance (100 divided by the number of explanatory variables) were considered non-relevant for interpretation (30). Considering the 12 variables tested, in this case, the relative influence threshold was 8.33%.

Regarding social perception, questions addressed abandonment causes and the areas and years in which the phenomenon was evident in the department of San Martín. Focus was placed on producers and their technical advisors. For this purpose, a pilot study was conducted and a qualitative online survey was administered through a Google Form. A total of 50 producers and/or advisors from San Martín were contacted through different social networks. The survey consisted of five open and closed questions on (1) major drivers of rural abandonment in the department of San Martín (considering depth of the water table, crop type, population density, access to road network, and water and labor availability), (2) additional environmental or socio-economic and cultural factors impacting the abandonment process; (3) localities with abandoned agricultural plots, (4) localities in which this process occurred with greater intensity between 2002 and 2020, and (5) the time in which the process of abandonment was intensified (dividing the period under analysis into three equal parts: 2002-2008, 2009-2014 or 2015-2020). The surveys were analyzed using descriptive statistics and compared with the spatial analysis.

Results

Rural abandonment maps derived from the four ΔNDVI thresholds found values of overall accuracy between 0.70 and 0.77 (table 2). The differences between these values lay in the accuracy with which abandoned and cultivated land was identified. At one extreme, the map derived from the threshold given by the Jenks Natural Breaks function correctly classified 63% of sample points identified as abandoned land (producer's accuracy 0.63), while it misclassified 29% of sample points identified as cultivated land (producer's accuracy 0.71). On the opposite, the map derived from the threshold defined as 2σ, correctly classified 36% of sample points identified as abandoned land (producer's accuracy 0.36), with 95% of the sample points identified as cultivated land correctly classified (producer's accuracy 0.95). Considering this paper aims to detect rural abandonment in the study area, the Jenks Natural Breaks function was selected as ΔNDVI threshold.

Table 2. Total, producer's, and user's accuracy associated with the four rural abandonment maps derived from assessed ΔNDVI thresholds: Jenks Natural Breaks (NB), 1σ, 1.5σ y 2σ.

Tabla 2. Exactitud total, del productor y del usuario asociadas a los cuatro mapas de abandono rural generados a partir de los umbrales de ΔNDVI analizados: Quiebres Naturales de Jenks (NB), 1σ, 1.5σ y 2σ.

ΔNDVI threshold	Total accuracy	Producer's accuracy			User´s accuracy		
		Abandoned land	Cultivated land	Not cultivated land	Abandoned land	Cultivated land	Not cultivated land
NB	0.70	0.63	0.71	0.84	0.50	0.82	0.79
1σ	0.75	0.50	0.86	0.84	0.61	0.80	0.81
1.5σ	0.77	0.42	0.93	0.84	0.71	0.78	0.79
2σ	0.77	0.36	0.95	0.84	0.74	0.77	0.79

According to the selected rural abandonment map, abandoned land increased by 92% during the study period. This was calculated considering the 13,492 ha in the reference map which was incremented to 25,869 ha in the 2020 rural abandonment map (table 3, page 42). Four areas concentrate abandoned land in north, central-west, central-east, and south San Martín (figure 2, page 42).

Table 3. Area (ha) for each land use category in the reference map (Mendoza productive map elaborated by IDR) and in the rural abandonment map (detected with NDVI difference technique). Variations are indicated in %.

Tabla 3. Área (ha) para cada categoría de uso de suelo en el mapa de referencia (mapa productivo de Mendoza elaborado por el IDR) y en el mapa de abandono rural (detectado con la técnica de diferencia del NDVI). Se indican los cambios (%) entre ellos.

Land use category	Reference map	Rural abandonment map	Changes (%)
Abandoned land	13,492	25,869	+92
Cultivated land	42,397	30,169	-29
Non-cultivated land	11,941	11,791	-1

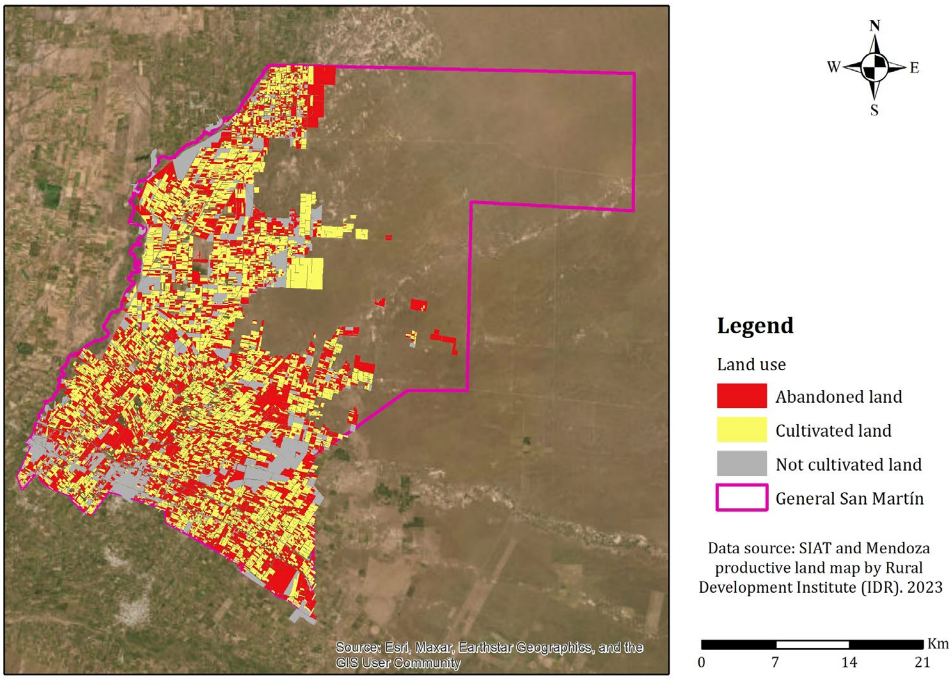


Figure 2. Rural abandonment map for the study area in 2020.

Figura 2. Mapa de abandono rural para el área de estudio en 2020.

The spatial analysis indicated that the Euclidean distance to national and provincial roads, crop type, percentage of households with unsatisfied basic needs (UBN), distance to wells and irrigation canals, and density of canals and groundwater extraction wells mostly explained the spatial distribution of abandoned land in the study area (table 4, page 43). The model explained 24.62% of the observed variability, probably due to the complexity of the analyzed problem. Distance to roads indicates accessibility to agricultural plots and accounts for management and harvesting difficulties. The crop would be linked to production profitability and local, national, and international market incentives and disincentives. The percentage of UBN households provides idea of structural poverty conditions in which some producers or their employees live (*e.g.*, contractors who work and live on the farm and receive a share of production profit), as well as households of local people working in companies, especially during harvest time. Finally, the four variables associated with irrigation canals and groundwater extraction wells account for irrigation availability. It should be noted that, geographically, San Martín largely covers the eastern boundary of the northern oasis of Mendoza.

Table 4. Relative influence of explanatory variables for a BRT model developed with a tree complexity of 3, a learning rate of 0.005, and a bag fraction of 0.5. Explained deviance was 24.62%

Tabla 4. Influencia relativa de las variables explicativas para el modelo de BRT desarrollado con complejidad de árbol de 3, tasa de aprendizaje de 0,005 y *bag fraction* de 0,5. La devianza explicada fue del 24,62%.

Explanatory variable	Relative influence
Distance to roads	14.03
Type of crop	13.41
% Households with unsatisfied basic needs	12.86
Distance to wells	11.34
Channel density	10.78
Well density	10.63
Distance to channels	8.80
Elevation	6.32
Storm damage	4.29
Population density	4.05
% Working-age population with only primary education	3.35
Aridity index	0.15

Twenty-seven out of 50 producers and/or advisors completed the survey. Concerning the most important drivers of land abandonment in San Martín, 85% of the respondents pointed to irrigation availability, 81% to labor availability, and 67% to crop type, in agreement with the spatial analysis. When asked what other biophysical or socio-economic and cultural factors impact the abandonment process, 63% of the respondents pointed to a lack of profitability. In addition, 15% indicated difficulties associated with generational changes (in the words of the interviewees "(...) aging of the vine-growers and lack of desire of the new generations to continue with the activity"), 15% related to the regional economy and another 15% associated with insecurity (robberies, vandalism, and theft). When respondents were asked to indicate which districts in San Martín currently had abandoned plots, 74% indicated Montecaseros, 56% El Divisadero and 48% El Ramblón. Particularly, regarding the process between 2002 and 2020, 77% of respondents indicated Montecaseros, 58% El Ramblón and 46% El Divisadero. Data from spatial analysis and surveys point to Montecaseros and El Ramblón as two districts with intermediate evidence of rural abandonment (figure 3, page 44). Nueva California district was identified by some respondents, but showed strong evidence of rural abandonment in the spatial analysis. Conversely, El Divisadero was noted by numerous respondents but showed less evidence of abandonment in the spatial analysis. It is worth noting that southern districts, where a large amount of abandoned land was detected according to the rural abandonment map, were slightly mentioned by respondents. Finally, 65% of respondents indicated that rural abandonment has intensified in the last period, 2015-2020.

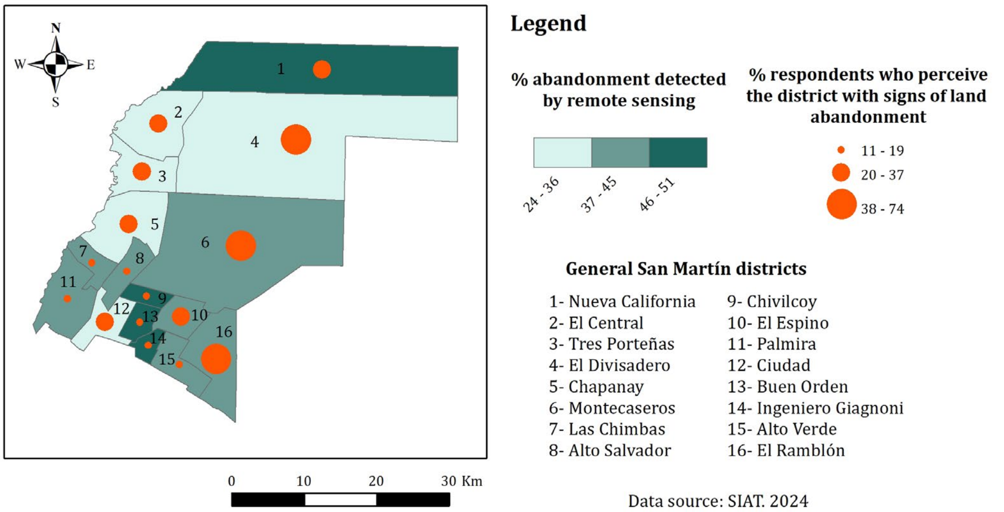


Figure 3. Location of rural abandonment in the study area according to remote sensing and respondents perception.

Figura 3. Localización del abandono rural en el área de estudio según la teledetección y la percepción de los encuestados.

Discussion

Rural abandonment is a global phenomenon associated with biophysical, socio-economic, and socio-productive causes (35, 40). In this respect, our results are in line with other studies associating this phenomenon with soil characteristics, topography and accessibility (12), the presence of soils with agricultural potential and agricultural subsidies (9), topography, accessibility, tractor and cropland density (30), crop yields and accessibility (33), physical environmental conditions, accessibility and global market pressures (3), equipment and materials costs and property taxes (24) or lack of state support, lack of occupation, demotivation of young people and lack of educational centers (31). In the study area, coincidences were found for both sources of information examined and also with these previous studies. According to the spatial analysis, rural abandonment would be associated with accessibility, type of crop, vulnerable living conditions of the local population, and availability of irrigation water. Whereas, the study of perception pointed to irrigation water and labor availability, type of crop, and the lack of profitability as major causes. Crop type is related to both profitability and agricultural management.

Regarding the territorial dimension, the NDVI difference technique showed that abandonment had increased in the analysed period at an alarming rate. All respondents acknowledged rural abandonment in the study area, recently intensified and associated with different districts in the department. The spatial pattern of rural abandonment showed some divergences between satellite image analysis and respondent perceptions. In some cases, both sources of information associated the process of agricultural land abandonment with different districts. It is worth noting that the sector where the greatest divergences were found (the south) overlaps with a zone defined by intense urban and industrial land uses in a matrix of cultivated areas. Two main, fast-growing cities in the department are located there: Ciudad de San Martín and Palmira. Moreover, the sector is crossed by National Route 7, one important commercial and tourist highway in the country, connecting the Atlantic and the Pacific oceans and integrating the Central Bioceanic Corridor. We could hypothesize that the development of this area interferes with the perception of rural abandonment. Although remote sensing analysis showed moderate to high levels of abandonment in south San Martín, respondents associated the phenomenon with locations farther away from these urban centers.

Remotely sensed information provides up-to-date databases supporting land management. Regarding rural abandonment, early detection of this type of change is particularly relevant to prevent and mitigate land degradation, which becomes the most efficient option. Thus, starting from an updated LULC map, detecting and monitoring changes may simplify and speed up the analysis (26). The methodology of LULC change detection used in this study resulted in a rapid application and easy implementation and interpretation. Compared with a previous study detecting abandoned land in a sector of the north oases (15), the implemented methodology could enable a larger-scale monitoring of the process. Therefore, the provided information could be useful for local authorities (among other social actors) for detecting the most compromised districts, speeding up the diagnosis, an invaluable phase of land use policy-making.

Rural abandonment becomes more complex in drylands, naturally vulnerable to degradation processes. In these regions, abandonment implies a loss of productive land and the entire associated technological system that is unlikely to be replaced due to the limited availability of water resources. Food security and local livelihoods are seriously at risk (9, 12). In this context, the complementation of quantitative data on landscape change with the perception of the local community is highly relevant and leads to understanding views, beliefs, and decisions of different social actors. This, in turn, is key to formulating public policies addressing processes of landscape change (14). On the other hand, studying the perception of social actors promotes a better understanding and interpretation of changes, enriches the explanation of driving factors, and leads to a comprehension of the complexity of the socio-economic and cultural context in which they occur (14, 16, 32). The incorporation of local community knowledge in decision-making contributes to strengthening alliances between the social actors involved, optimizing land-use planning processes with a holistic and long-term vision in pursuit of sustainable use of productive resources.

Conclusions

In the department of San Martín, located east of the northern oasis of Mendoza (Argentina), cropland abandonment increased by 92% between 2002 and 2020. As in other places around the world, this phenomenon is multi-causal. Accessibility, crop type, vulnerable living conditions of the local population, availability of irrigation water and labor, and lack of profitability constitute main drivers for land abandonment. Addressing this complex problem needs to integrate different sources of information and skills to develop public land-use planning policies that promote sustainable local development. The loss of productive land in drylands threatens food security, determining the urgency of preserving existing cultural and productive resources. The multidisciplinary approach implemented in this study used different methodologies and sources of information enabling a better understanding of the environmental problem. Moreover, it allowed defining differences between the perception of local social actors and the results derived from spatial analysis tools. Understanding and managing territorial complexity from an interdisciplinary perspective is essential to contributing knowledge to the local social reality.

References

1. Abraham, E. M. 2002. Lucha contra la desertificación en las Tierras Secas de Argentina; el caso de Mendoza. In El agua en Iberoamérica; De la escasez a la desertificación. ed A. Fernández Cirelli, E. M. Abraham. p. 27-44. CYTED XVI Prólogo - Editores.
2. Abraham, E.; Rubio, M. C.; Rubio, C.; Soria, D. 2017. Análisis del subsistema físico-biológico. In Ordenar el territorio. Un desafío para Mendoza. p. 36-106. EDIUNC. Colección Territorios.
3. Beilin, R.; Lindborg, R.; Stenseke, M.; Pereira, H. M.; Llausàs, A.; Slätmo, E.; Cerqueira, Y.; Navarro, L.; Rodrigues, P.; Reichelt, N.; Munro, N.; Queiroz, C. 2014. Analysing how drivers of agricultural land abandonment affect biodiversity and cultural landscapes using case studies from Scandinavia, Iberia and Oceania. Land use policy. 36: 60-72.
4. Benjamin, K.; Bouchard, A.; Domon, G. 2007. Abandoned farmlands as components of rural landscapes: An analysis of perceptions and representations. Landsc Urban Plan. 83(4): 228-244.
5. Cabrera, Á. L. 1971. Fitogeografía de la República Argentina. Boletín de la Sociedad Argentina de Botánica. XIV(1-2): 1-42.

6. Congalton, R. G. 1991. A review of assessing the accuracy of classifications of remotely sensed data. Remote Sens Environ. 37(1): 35-46.
7. DACC (Dirección de Agricultura y Contingencias Climáticas). Daños Zona Este. www.contingencias.mendoza.gov.ar
8. Dedman, S.; Officer, R.; Brophy, D.; Clarke, M.; Reid, D. G. 2017. Advanced spatial modeling to inform management of data-poor juvenile and adult female rays. Fishes. 2(3): 1-22.
9. Díaz, G. I.; Nahuelhual, L.; Echeverría, C.; Marín S. 2011. Drivers of land abandonment in Southern Chile and implications for landscape planning. Landsc Urban Plan. 99(3-4): 207-217.
10. Dougill, A. J.; Twyman, C.; Thomas, D. S. G.; Sporton, D. 2002. Soil degradation assessment in mixed farming systems of southern Africa: use of nutrient balance studies for participatory degradation monitoring. Geogr J. 168(3): 195-210.
11. Elith, J.; Leathwick, J. R.; Hastie, T. 2008. A working guide to boosted regression trees. Journal of Animal Ecology. 77(4): 802-813.
12. Gellrich, M.; Zimmermann, N. E. 2007. Investigating the regional-scale pattern of agricultural land abandonment in the Swiss mountains: A spatial statistical modelling approach. Landsc Urban Plan. 79(1): 65-76.
13. Giordano, R.; Liersch, S. 2012. A fuzzy GIS-based system to integrate local and technical knowledge in soil salinity monitoring. Environmental Modelling & Software. 36: 49-63.
14. González-Puente, M.; Campos, M.; McCall, M. K.; Muñoz-Rojas, J. 2014. Places beyond maps; integrating spatial map analysis and perception studies to unravel landscape change in a Mediterranean mountain area (NE Spain). Applied Geography. 52: 182-190.
15. Guida-Johnson, B.; Sales, R. G.; Esteves, M. 2020. Presión de la expansión urbana sobre territorios rurales de tierras secas irrigadas de Mendoza. Reflexiones para el ordenamiento territorial. Revista de la Asociación Argentina de Ecología de Paisajes. 9(1): 165-169.
16. Hearn, K. P.; Alvarez-Mozos, J. 2021. A diachronic analysis of a changing landscape on the duero river borderlands of Spain and Portugal combining remote sensing and ethnographic approaches. Sustainability. 13(24): 13962.
17. INDEC. 2013. Censo Nacional de Población, Hogares y Viviendas 2010, procesado con Redatam+SP. https://redatam.indec.gob.ar/argbin/RpWebEngine.exe
18. INDEC. 2022. Censo Nacional de Población, Hogares y Viviendas 2022. https://censo.gob.ar/index.php
19. Izquierdo, A. E.; Grau, H, R. 2009. Agriculture adjustment, land-use transition and protected areas in Northwestern Argentina. J Environ Manage. 90: 858-865.
20. Jarvis, A.; Reuter, H. I.; Nelson, A.; Guevara, E. 2008. Hole-filled seamless SRTM data V4. International Centre for Tropical Agriculture (CIAT). http://srtm.csi.cgiar.org
21. Jiang, L.; Jiapaer, G.; Bao, A.; Li, Y.; Guo, H.; Zheng, G.; Chen, T.; De Maeyer, P. 2019. Assessing land degradation and quantifying its drivers in the Amudarya River delta. Ecol Indic. 107: 105595.
22. Karakani, E. G.; Malekian, A.; Gholami, S.; Liu, J. 2021. Spatiotemporal monitoring and change detection of vegetation cover for drought management in the Middle East. Theor Appl Climatol. 144(1-2): 299-315.
23. Koutroulis, A. G. 2019. Dryland changes under different levels of global warming. Science of the Total Environment. 655: 482-511.
24. Kuntz, K. A.; Beaudry, F.; Porter, K. L. 2018. Farmers' perceptions of agricultural land abandonment in rural western New York state. Land (Basel). 7(4): 1-11.
25. Lu, D.; Mausel, P.; Brondízio, E.; Moran, E. 2004. Change detection techniques. Int J Remote Sens. 25(12): 2365-2407.
26. Lunetta, R. S.; Knight, J. F.; Ediriwickrema, J.; Lyon, J. G.; Worthy, L. D. 2006. Land-cover change detection using multi-temporal MODIS NDVI data. Remote Sens Environ. 105(2): 142-154.
27. Mancino, G.; Nolè, A.; Ripullone, F.; Ferrara, A. 2014. Landsat TM imagery and NDVI differencing to detect vegetation change: Assessing natural forest expansion in Basilicata, southern Italy. IForest. 7(2): 75-84.
28. Meyer, M. A.; Früh-Müller, A. 2020. Patterns and drivers of recent agricultural land-use change in Southern Germany. Land use policy. 99: 104959.
29. Michener, W. K. 1997. Quantitatively evaluating restoration experiments: research design, statistical analysis, and data management considerations. Restor Ecol. 5(4): 324-337.
30. Müller, D.; Leitão, P. J.; Sikor, T. 2013. Comparing the determinants of cropland abandonment in Albania and Romania using boosted regression trees. Agric Syst. 117: 66-77.
31. Muñoz-Rios, L. A.; Vargas-Villegas, J.; Suarez, A. 2020. Local perceptions about rural abandonment drivers in the Colombian coffee region: Insights from the city of Manizales. Land use policy. 91: 104361.
32. Pătru-Stupariu, I.; Tudor, C. A.; Stupariu, M. S.; Buttler, A.; Peringer, A. 2016. Landscape persistence and stakeholder perspectives: The case of Romania's Carpathians. Applied Geography. 69: 87-98.
33. Prishchepov, A. A.; Müller, D.; Dubinin, M.; Baumann, M.; Radeloff, V. C. 2013. Determinants of agricultural land abandonment in post-Soviet European Russia. Land use policy. 30(1): 873-884.
34. R Development Core Team. 2019. R: A language and environment for statistical computing.

35. Rey Benayas, J. M.; Martins, A.; Nicolau, J. M.; Schulz, J. J. 2007. Abandonment of agricultural land: an overview of drivers and consequences. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2(57): 1-14.
36. Rojas, F.; Rubio, C.; Rizzo, M.; Bernabeu, M.; Akil, N.; Martín F. 2020. Land use and land cover in irrigated drylands: A long-term analysis of changes in the Mendoza and Tunuyán River basins, Argentina (1986-2018). Appl Spat Anal Policy. 13(4): 875-899.
37. Salvia, R.; Quaranta, V.; Sateriano, A.; Quaranta, G. 2022. Land resource depletion, regional disparities, and the claim for a renewed "sustainability thinking" under early desertification conditions. Resources. 11(3): 28.
38. SIAT (Sistema de Información Territorial y Ambiental). 2013. Índice de aridez. www.siat.mendoza.gov.ar
39. SIAT (Sistema de Información Territorial y Ambiental). 2014. Ejes de calles. www.siat.mendoza.gov.ar
40. Smaliychuk, A.; Müller, D.; Prishchepov, A. V.; Levers, C.; Kruhlov, I.; Kuemmerle, T. 2016. Recultivation of abandoned agricultural lands in Ukraine: Patterns and drivers. Global Environmental Change. 38: 70-81.
41. Sohl, T. L. 1999. Change analysis in the United Arab Emirates: An investigation of techniques. Photogramm Eng Remote Sensing. 65(4): 475-484.
42. UNCCD (United Nations Convention to Combat Desertification). 2014. The land in numbers. Livelihoods at a tipping point. 1-22. https://www.unccd.int/sites/default/files/documents/Land_In_Numbers_web.pdf
43. Verón, S. R.; Lizana, P. R.; Maggi, A. 2022. Cartografía de las tierras secas en Argentina, índice de aridez en el periodo 1981-2020.
44. Vignoni, A. P.; Peralta, I. E.; Abraham, E. M. 2023. Fragmented areas due to agricultural activity: native vegetation dynamics at crop interface (Montecaseros, Mendoza, Argentina). Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 46-60. DOI: https://doi.org/10.48162/rev.39.108.

Acknowledgement

This work was supported by the Universidad Nacional de Cuyo under Grant SIIP 2019 Tipo 1 M079. The authors thank anonymous reviewers whose valuable comments and suggestions helped improve the manuscript.

Agrochemical characterization of *Vitis labrusca* grape pomace and its effect on a soil-plant system

Caracterización agroquímica del orujo de uva de *Vitis labrusca* y su efecto sobre el sistema suelo-planta

María Inés Troncozo [1*], Hugo Merani Victor [2], Alejandra Bárcena [3], María Gabriela Cano [3], María Florencia Vianna [1]

Originales: *Recepción:* 19/08/2023 - *Aceptación:* 28/02/2024

Abstract

This study characterized the agrochemical properties of *V. labrusca* grape pomace (GP) and evaluated the effect on the rhizobacteria *Azospirillum brasiliense* and horticultural crops, determining safety as fertilizer and/or mulching. Two first bioassays were performed with the GP at different concentrations evaluating toxicity on *A. brasiliense*, and on tomato and lettuce seeds. A third bioassay evaluated GP mulching effects on tomato and lettuce plants growing with amounts varying between 20 and 80 t ha^{-1}. Agrochemical characterization showed that GP is rich in potassium and phosphorus, with a low content of Na^+ salts (SAR < 15). The GP at 2.5% (w v^{-1}) significantly increased survival rates of N_2-fixing rhizobacteria. Results on seed germination revealed lettuce was more susceptible to increasing GP concentrations. The application of 20 t ha^{-1} of GP in greenhouse experiments increased lettuce and tomato root biomass. Furthermore, the aerial part of tomato showed no toxicity symptoms. These results open the possibility of considering *V. labrusca* GP as mulching without prior treatment in tomato crops.

Keywords

agrowaste • revalorization • macronutrients • *Azospirillum brasiliense* • tomato • lettuce

1 Universidad Nacional de La Plata (UNLP). Facultad de Ciencias Naturales y Museo. Instituto de Botánica Carlos Spegazzini. 122 y s/n. 1900 La Plata. Argentina.
[*] mi.troncozo@conicet.gov.ar

2 Universidad Nacional de La Plata (UNLP). Facultad de Cs. Agrarias y Forestales. Centro de Investigación de Suelos para la Sustentabilidad Agropecuaria y Forestal (CISSAF) 119 y 60. 1900 La Plata. Argentina.

3 Universidad Nacional de La Plata (UNLP). Instituto de Fisiología Vegetal (INFIVE). CCT-La Plata-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Diag. 113 y 61. CC 327. 1900 La Plata. Argentina.

Resumen

Este estudio tuvo como objetivo caracterizar las propiedades agroquímicas del orujo de uva (GP) de *V. labrusca* y evaluar su efecto sobre *Azospirillum brasiliense* y cultivos hortícolas para determinar su uso seguro como fertilizante y/o mulching. Los bioensayos se realizaron con el GP a diferentes concentraciones para evaluar su toxicidad sobre *A. brasiliense* y semillas de tomate y lechuga. Para evaluar el efecto de GP sobre el crecimiento de plantas de tomate y lechuga se aplicaron diferentes dosis como mulching en el rango de 20-80 t ha^{-1}. La caracterización agroquímica mostró que GP es rico en potasio y fósforo; y tiene bajo contenido de sales de Na^+ (SAR < 15). El GP al 2,5% (p v^{-1}) fue responsable de un aumento significativo en la tasa de supervivencia de las rizobacterias fijadoras de N_2. Los resultados de la germinación de las semillas revelaron que la lechuga fue más susceptible al aumento de las concentraciones de GP. La aplicación de 20 t ha^{-1} de GP en los experimentos de invernadero incrementó la biomasa radicular de lechuga y tomate. Además, la parte aérea del tomate no presentó síntomas de toxicidad. Estos resultados abren la posibilidad de considerar el aprovechamiento de *V. labrusca* GP como mulching, sin utilizar ninguna tecnología de tratamiento antes de su aplicación al suelo en cultivos de tomate.

Palabras claves

residuo agronómico • revalorización • macronutrientes • *Azospirillum brasiliense* • tomate • lechuga

Introduction

The wine industry is one of the oldest and most important in the world. In 2019, wine production was estimated at around 258 Mhl (27). Grapes for winemaking belong to the Genus *Vitis* sp, being *V. vinifera* and *V. labrusca* the two main producing species (20). *V. vinifera* is cultivated in arid soils, poor in organic matter, while the hybrid *V. labrusca* originated in eastern USA, grows in different edaphoclimatic regions and is highly resistant to diseases (18, 24). In South America, mainly in Argentina and Brazil, *V. labrusca* cultivated areas have increased in the last decade (24, 40).

Annually, the wine industry produces 13 x 10^6 t of solid waste or by-products worldwide, with grape pomace (GP) being the most abundant (10-13 Mt per year). In terms of circular economy, GP could generate new products of different values (7, 21), closing the cycle as mulching or organic fertilizer due to its lignocellulosic nature (8). Mulching improves soil structure, keeps moisture, moderates temperature, and increases organic matter (OM), returning soil nutrient cycling environments to pristine soil patterns (25). On the other hand, when the agronomic purpose is to increase crop yield, organic fertilizers of plant origin constitute an interesting agroecological alternative for intensive crops like vegetables, flowers, and orchards (22). Nevertheless, a proper agronomic management program incorporating lignocellulosic by-products, such as GP, needs to characterize the product and establish an appropriate use. Unlike the well-known physicochemical characteristics of *V. vinifera* GP (6, 16, 34), the relevant agrochemical properties of *V. labrusca* GP have not been studied yet. Direct incorporation of *V. vinifera* GP into the soil could negatively affect plant growth due to inhibitory compounds or given competition between soil microorganisms for essential nutrients such as nitrogen (6). However, this study only considers GP physicochemical properties, leaving aside other sensitive parameters in the soil-plant systems.

Biological indicators are living organisms, such as plants, animals, and/or microorganisms exploited to detect toxic substances in terrestrial and aquatic ecosystems (45). The effect of agrowastes on the soil-plant system can be evaluated by lettuce (*Lactuca sativa*) and tomato (*Solanum lycopersicum*) as biological indicators, as well as by the free-soil nitrogen-fixing bacterium *Azospirillum brasiliense* (14, 36, 37, 41). The combination of agrochemical characterization together with biological indicators enables the development of appropriate agronomic management programs (22).

This study characterized the agrochemical properties of *V. labrusca* var. *Isabella* GP and evaluated toxicity effects on *A. brasiliense*, tomato and lettuce determining GP safety as fertilizer or/and mulching.

MATERIAL AND METHODS

Study area and GP sampling

The study considered twenty-four ha of *V. labrusca* var *Isabella* at "Cooperativa de la Costa'' wine-cellar located in Berisso, Buenos Aires, Argentina (34°53'22.79" S; 57°49'21.11" W (28). The soil is Rendzic Leptosol (11) with an A-horizon of 20-40 cm and a high content of well-humified organic matter, under which there is an AC or C-horizon constituted by layers of small shells (1). In 2013, the winery produced 350 hL of wine and a total of 12 t of GP, representing 25% of grape total weight (29).

Samplings were carried out in 2015, 2016 and 2017 seasons. Each year, 10 random GP samples were collected immediately after grape pressing and blended to obtain a compound sample. One part of the sample was separated to determine moisture content. The rest was oven-dried at 60°C to constant weight and then divided into sub-samples. These were stored in hermetically sealed in containers until GP physicochemical characterization. Part of fresh material from the 2017 campaign was separated and stored at -20°C for subsequent bioassays.

Agrochemical GP characterization

Ashes and OM were determined by calcination (6). Percentage contents of organic carbon (C) and total nitrogen (N) were determined after Walkley and Black (1934) and micro-Kjeldahl methods (5) respectively, and the C/N ratio was calculated. Total phosphorus (P) was determined by the yellow vanadate-molybdate method (19). Extractable and exchange cations were obtained by the saturated paste extraction method and the Ammonium acetate method 1N pH7, respectively (35). Calcium (Ca^{+2}) and Magnesium (Mg^{+2}) were estimated by complexometry with 0.02 N EDTA; Sodium (Na^{+}) and Potassium (K^{+}) by flame photometry. Sodium adsorption ratio (SAR) was calculated using the following equation (31) (eq. 1).

$$\mathbf{SAR = (Na^{+}\ ext) / [\ (Ca^{+2}\ ext + Mg^{+2}\ ext /2]^{1/2}} \qquad (1)$$

The Mg^{+2}/K^{+}, Ca^{+2}/Mg^{+2}, and Ca^{+2}/K^{+} ratios were calculated using the obtained values for individual exchangeable cations (17). Cation exchange capacity (CEC) was determined by steam distillation and percentage base saturation (S) was calculated by eq. 2 (33).

$$\mathbf{S\ (\%) = (\Sigma\ exchangeable\ cations/CEC)\ {*}100} \qquad (2)$$

Electric conductivity (EC) and pH values were measured in the aqueous soluble fraction (1:5 ratio) by conductometry and potentiometry, respectively (19). Moisture content (%) was determined by oven-drying samples at 60°C until constant weight and calculating the difference between wet and dry weight. Water-soluble phenols (WSP) were determined as described by Osono and Takeda (2001). Three technical replicates were used to determine each physicochemical parameter. Values were averaged to obtain a unique value for each year. Mean, range and t variance were estimated with three annual values. The classification criterion proposed by Fernández Linares *et al.* (2006) and Havlin *et al.* (1999) allowed interpretation.

Bioassays with GP application

Three bioassays determined GP safety as fertilizer or/and mulching: a) GP toxicity evaluation on a N fixing microorganism (*A. brasiliense*), b) GP toxicity evaluation on germination of tomato and lettuce seeds; and c) GP effect on growth parameters of tomato and lettuce plants.

a)- GP toxicity evaluation on a N fixing microorganisms (*A. brasiliense*)

GP effects on survival of *A. brasiliense* CECT 590 T were evaluated according to

Saparrat *et al.* (2010). A bacterial suspension (100 µl) was inoculated in 900 µl of the GP sterilized by filtration (0.22 µm millipore membrane) at different concentrations: 2.5, 3.5, 5, and 10% (w v^{-1}). Controls were made using sterile distilled water and 4 replicates per treatment were utilized. Cultures were grown at 28°C and 150 rpm for 24 h. Colony-forming units (CFU mL^{-1}) were estimated by the dilution and plating method using the selective medium Congo red. The data were transformed to logarithms and analyzed by ANOVA and a Tukey test ($p < 0.05$).

b)- GP toxicity evaluation on germination of tomato and lettuce seeds

GP phytotoxicity was evaluated after the effects observed on germination and root growth of lettuce and tomato according to Tiquia *et al.* (1996) with modifications. Seeds of each species were placed on filter paper inside Petri dishes (9 mm diameter) in contact with 3 ml of the GP at different concentrations: 2.5, 5, 10, and 20% w v^{-1}. Negative and positive controls consisted of paper soaked with sterile distilled water and 1 M of a $CuSO_4$ solution, respectively. Four replicates per treatment were incubated at 25°C for 7 days in the dark.

Number of germinated seeds and root length (mm) were determined. Relative germination percentage (G) (eq. 3), relative root length (RL) (eq. 4), and germination index (GI) (eq. 5) were calculated using the following equations:

$$\textbf{G (\%) = (number of germinated seeds at X GP concentration/ number of seeds germinated in negative control) *100} \quad (3)$$

$$\textbf{RL (\%) = (mean root length at X GP concentration/ mean root length in negative control) *100} \quad (4)$$

$$\textbf{IG = (G * RL) / 100} \quad (5)$$

A 2 mm primary root defined seed germination. The data obtained were analyzed by ANOVA and Tukey test ($p < 0.05$). Percentual data were transformed using arc sen $\sqrt{p}$, before statistical analysis.

c) GP effect on growth of tomato and lettuce plants

The effect of GP on lettuce and tomato plants was determined using ten plants per treatment and according to Sampedro *et al.* (2004) with modifications. Seedlings with three expanded leaves were placed in 5 L pots filled with a mixture of soil and sand in a 1:1 ratio (v v^{-1}). GP mulching was added to the pots at different doses: 20, 40, and 80 t h^{-1} (wet weight equivalent to 60, 120 and 240 g of GP per pot). The plants were grown for 1 month in a greenhouse and periodically watered. Pots without GP were set up as control. The number of expanded leaves per plant was estimated by direct counting. Leaf greenness (GrI) was measured using a portable chlorophyll meter (SPAD-502, Minolta Corp. Japan), randomly selecting three expanded leaves per plant. Total leaf area per plant was captured by photography and analyzed using J image software (38). Plants were harvested and soil adhered to roots was washed with running water. The aerial part of the plants and roots were oven-dried at 60°C to constant weight determining aerial and root dry biomass. The data were analyzed for each plant species comparing dose effects. A Kruskal-Wallis test and a non-parametric multiple contrast as *post hoc* test ($p < 0.05$) analyzed the number of expanded leaves. The remaining data were analyzed by ANOVA and Tukey test ($p < 0.05$).

Results

Agrochemical GP characterization

The C/N ratio < 25 suggested an equilibrate decomposition of OM. Among extractable cations, GP showed high levels of K^+ and low Na^+, high CEC (> 40), and a varied macronutrient supply (exchangeable cations, high content of K^+, followed by Ca^{+2}, and low Mg^{+2}). The GP exhibited a high base saturation index (S > 40), high EC (> 4 dS m^{-1}), extremely acidic pH, and high hygroscopicityy (> 70% humidity, table 1, page 52).

Table 1. Physicochemical characteristics of *V. labrusca* var. *isabella* GP.

Tabla 1. Características fisicoquímicas del OU de *V. labrusca* var. *isabella*.

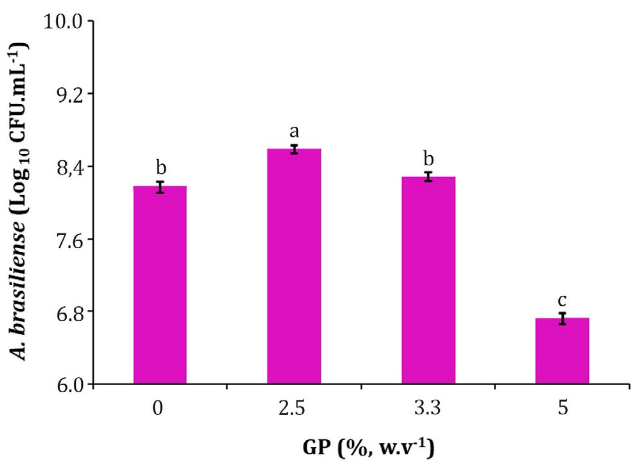
Parameter	Mean	Range	VC[1]
OM (g kg^{-1})	9.3	9.1-9.4	0.2
Ash (%)	7.5	6.4-9.1	18.9
TOC (g kg^{-1})	4.71	3.42-5.51	2.39
TN (g kg^{-1})	0.21	0.2-0.22	0.58
C/N	22.4	16.6- 26.3	26.5
TP (g kg^{-1})	3.1	2.9-3.4	8.6
Ext K^+ (g kg^{-1})	37.6	27.7-51.7	33.3
Ext Na^+ (mg kg^{-1})	64	57-75	15.1
Ext Ca^{+2} (g kg^{-1})	4.4	3.7-5.0	15.0
Ext Mg^{+2} (g kg^{-1})	0.7	0.5-0.8	23.2
SAR[2]	1.3^{-3}	1.1^{-3}- 1.51^{-3}	14.7
Exch K^+ ($cmol_c$ Kg^{-1})	49.3	43.9-54.1	10.4
Exch Ca^{+2} ($cmol_c$ Kg^{-1})	7.2	6.6-7.6	7.2
Exch Mg^{+2} ($cmol_c$ Kg^{-1})	0.9	0.4-1.7	72.9
Exch Na^+ ($cmol_c$ Kg^{-1})	0.4	0.4-0.5	21.0
Exch Ca^{+2}/ Exch Mg^{+2}	10.47	4.5-16.5	57.8
Exch K^+/ Exch Ca^{+2}	6.9	5.8-7.6	14.2
Exch K^+/ Exch Mg^{+2}	75.9	25.8-124.5	65.0
CEC ($cmol_c$ Kg^{-1})	43.7	41.5-46.7	6.2
S[3] (%)	132.4	125.7-137.7	4.64
EC (dS m^{-1})	5.8	5.4-6.1	6.1
pH	3.7	3.6-3.7	0.7
Moisture (%)	79.2	78.1-80.1	1.3
WSP[4] (mg g^{-1})	74.0	73.4-75.4	1.5

1, Variance coefficient. 2, Sodium adsorption ratio. 3, Base saturation index. 4, Water-soluble phenols. Extractable (Ext) and interchange (Exch) cations. n= 3 annual samples.

1, Coeficiente de variación. 2, Radio de adsorción de sodio. 3, Índice de saturación de bases. 4, Fenoles hidrosolubles. Cationes extractables (Ext) e intercambiables (Exch). n= 3 muestras anuales.

GP toxicity evaluation on a N fixing microorganism (*A. brasiliense*)

GP concentrations showed significant differences in *A. brasiliense* growth (F= 684.50; p <0.001). No CFU of the diazotrophic bacteria was recovered at 10% GP concentration (figure 1).



Means followed by the same letter are not significantly different (Tukey test, p < 0.05).

Los datos son medias de cuatro réplicas ± D.E. Medias seguidas por la misma letra no presentan diferencias significativas (Test de Tukey, p < 0,05).

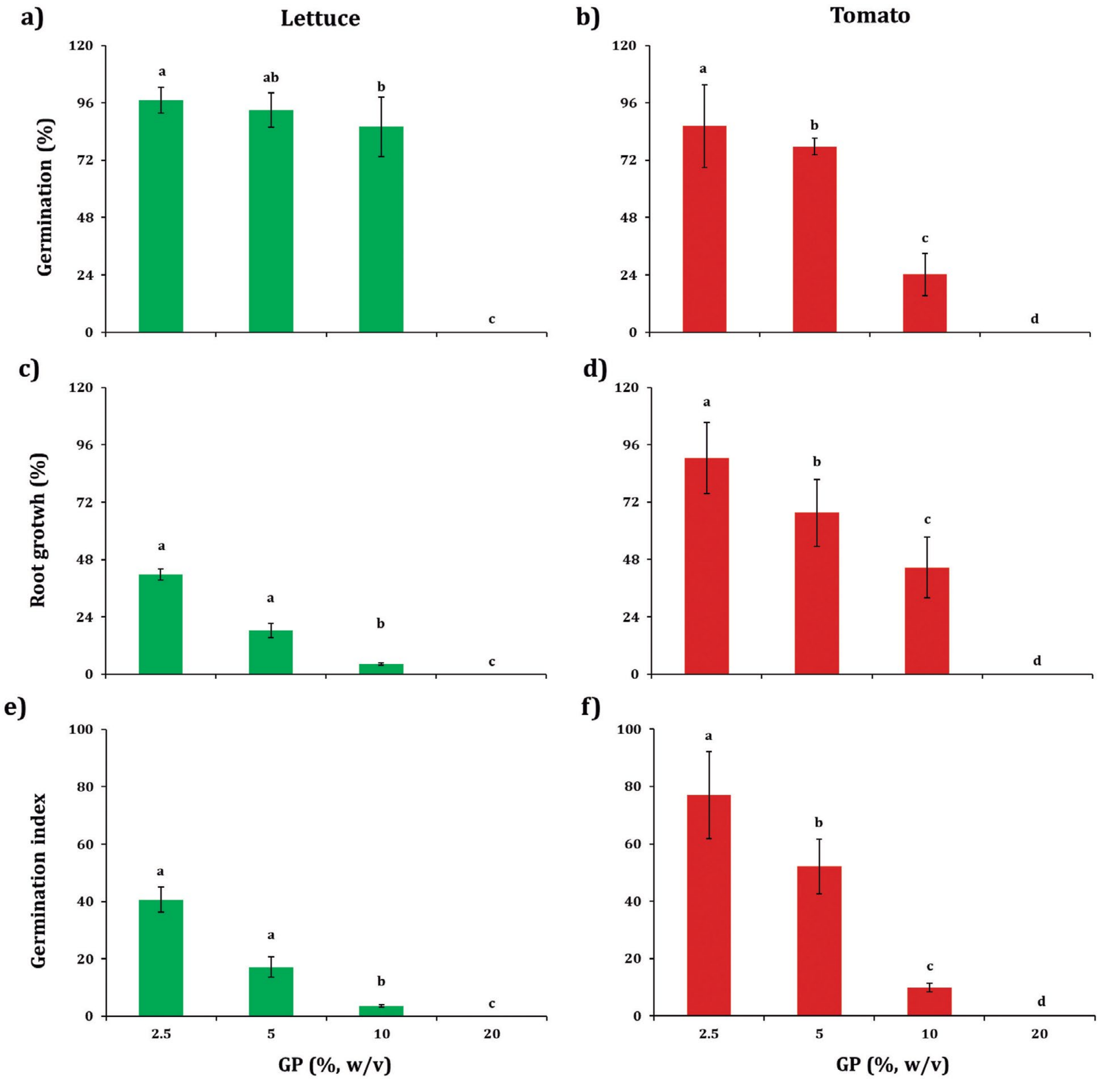
Figure 1. *A. brasiliense* growth after 24 h of incubation in several concentrations of GP (%, w v^{-1}). The data are means of four replicates ± S.D.

Figura 1. Crecimiento de *A. brasiliense* después de 24 h de incubación en distintas concentraciones de OU (%, p v^{-1}).

GP toxicity evaluation on germination of tomato and lettuce seeds

Significant differences were found in relative germination (G) (F= 86.04; p <0.001), relative root length (RL) (F= 583.56; p <0.001) and germination index (GI) (F= 348.69; p <0.001) when lettuce seeds were incubated at different GP concentrations. The G values at 2.5, 5 and 10% GP concentration equalled or exceeded 90%. The RL and GI, registered values below 50% indicating a toxic effect of GP at all concentrations evaluated (figure 2a, c, e).

For tomato, significant differences were found in G (F= 368.32; p <0.001), RL (F= 145.96; p <0.001) and GI (F= 278.02; p <0.001). The toxic effect in G, RL and GI increased with GP concentration (figure 2b, d, f).



Mean ± S.D. of four replicates followed by the same letter are not significantly different (Tukey test, p < 0.05).

Los datos son medias de cuatro réplicas ± D.E. Medias seguidas por la misma letra no presentan diferencias significativas (Test de Tukey, p < 0,05).

Figure 2. Effect of GP addition on germination, root length and germination index of lettuce and tomato plants.

Figura 2. Efecto de la adición del OU sobre la germinación, longitud radicular e índice de germinación de plantas de lechuga y tomate.

GP effect on growth of tomato and lettuce plants.

Significant differences were found in expanded leaf number (EL) (H= 8.54; *p*= 0.0259), greenness index (GrI) (F= 14.21; $p < 0.0001$), total leaf area (TLA) (F= 95.71; $p < 0.0001$), aerial dry weight (ADW) (F= 77.05; $p < 0.0001$) and root dry weight (RDW) (F= 40.54; $p < 0.0001$) of lettuce. The TLA, ADW and RDW significantly decreased with GP doses over 40%. The addition of 20 t ha^{-1} of GP as mulch showed an increase in RDW.

For tomato plants, significant differences were found in GrI (F= 6.72; *p*= 0.0038), TLA (F= 46.47; $p < 0.0001$), ADW (F= 21.00; $p < 0.0001$) and RDW (F= 30.54; $p < 0.0001$). The GP presented the highest toxic effect on ADW and TLA at doses of 40-80 t ha^{-1}. In contrast, GrI significantly increased at the maximum dose. Also, a non-toxic effect of GP was observed on RDW at 20 t ha^{-1} (table 2).

Table 2. Expanded leaf number (EL), greenness index (GrI), total leaf area (TLA), aerial dry weight (ADW) and root dry weight (RDW) of lettuce and tomato plants grown with increasing GP doses.

Tabla 2. Número de hojas expandidas (EL), Índice de verdor (GrI), Área foliar total (TLA), Biomasa seca área (ADW) y biomasa seca radicular (RDW) de plantas de lechuga y tomate en respuesta a diferentes dosis crecientes de OU.

Doses (t ha^{-1})	EL (n° $plant^{-1}$)		GrI (SPAD units)		TLA (cm^2 $plant^{-1}$)		ADW (g $plant^{-1}$)		RDW (g $plant^{-1}$)	
Lettuce										
0	11	a	28.2 ± 1.8	a	656.9 ± 85.3	a	3.6 ± 0.4	a	0.6 ± 0.1	b
20	9	b	19.8 ± 2.9	b	295.7 ± 18.9	b	2.3 ± 0.3	b	0.8 ± 0.1	a
40	9	b	23.3 ± 1.9	b	180.0 ± 30.7	c	1.0 ± 0.2	c	0.3 ± 0.0	c
80	9	b	22.1 ± 1.7	b	195.9 ± 41.7	c	1.0 ± 0.3	c	0.3 ± 0.0	c
Tomato										
0	11	A	31.2 ± 4.0	B	552.1 ± 57.2	A	9.1 ± 2.2	A	0.6 ± 0.1	B
20	11	A	31.2 ± 1.9	B	548.7 ± 43.9	A	9.5 ± 0.6	A	0.8 ± 0.1	A
40	10	A	36.3 ± 4.8	AB	275.7 ± 56.5	B	3.7 ± 1.1	B	0.4 ± 0.0	C
80	11	A	38.1 ± 3.5	A	295.6 ± 41.2	B	4.0 ± 1.8	B	0.4 ± 0.1	C

Data means ± S.D. of 10 replicates. For each row and vegetal species, different letters indicate significant differences ($p < 0.05$; Tukey test or Kruskal-Wallis test).

Los datos son medias de 10 réplicas ± D.E. Para cada fila y especie vegetal, diferentes letras indican diferencias significativas ($p < 0,05$; Test de Tukey o Kruskal-Wallis).

Discussion

This study found high OM contents in *V. labrusca* GP, as reported for *V. vinifera* and other agroindustrial by-products like solid olive mill "alperujo" (*Olea europaea* L.) and coffee pulp (2, 3, 4, 6). Unlike oil and coffee by-products, grape pomaces have high NT content and a C/N ratio nearly under the recommended limits for organic fertilizing (< 25) (6, 22).

Depending on the origin, commercial organic fertilizers have diverse amounts of essential nutrients (23). Bustamante *et al.* (2008) found 1.15 g kg^{-1} total P for *V. vinifera*, while in this study *V. labrusca* showed a higher total P content, resulting in a more attractive by-product for the agronomic industry.

While most organic fertilizers usually require EC values under 4 dS m^{-1}, the value obtained in this study was higher and considered detrimental to plant growth (22). However, the main salts found in *V. labrusca* GP are Ca^{+2} and/or Mg^{+2} chlorides and sulfates (SAR < 15). In this sense, the pomace could be considered a good alternative to animal manure causing soil disintegration due to concentrated Na^{+} salts (9, 10). Furthermore, *V. labrusca* pomace exhibited a CEC value similar to that of highly productive soils (>45), probably given by the available functional groups negatively charged (phenols, carboxylic acids, etc.) found in OM (15). Hence, soil addition of GP might reduce leaching, thus increasing essential cations availability (39).

Soils with a saturation base close to 100% exhibit alkaline pH (14). In this study, the pomace showed an elevated base saturation while being an acidic by-product, probably given to the presence of organic acids like malic and tartaric, common in grapes, that together with K+ and its effect over the diminished free tartaric acid, define by-product acidity (3). On the other hand, the high moisture content in this by-product (79.2%) is within the range reported for *V. vinifera* pomace and other agroindustrial wastes such as alperujo and coffee pulp ranging between 64-80% (3, 4).

Introducing OM into the system could differentially affect soil microorganisms and plants, depending on application dose and tolerance ranges (26, 41). *A. brasiliense* is a soil-free nitrogen-fixing bacteria producing several plant signalling molecules like phytohormones (auxins and gibberellins) (13, 32). The positive effect of GP on this diazotrophic bacteria has not been previously reported using other by-products (37).

When seeds were exposed to 10% GP concentration, primary roots showed a brownish colour in both plant species, probably given by cell necrosis caused by toxic compounds in GP or polymerization of compounds (chromophores) in root exudates as a defence response. A similar symptom was reported in tomato roots as an allelochemical effect of *Sicyos deppei* (Cucurbitaceae) (33). Some phenols act as allelochemical compounds related to polar narcosis (structural and functional alteration of cell membrane), oxidation uncoupling, alterations in electrophilicity, hydrophobicity, and dissociation and union of H in biomolecules (30, 44). Since *V. labrusca* pomace is rich in soluble phenolic compounds, these substances could be related to the mentioned root symptoms.

In the plant experiments, the stimulant effect of GP on root systems at the lowest dose could be due to greater nutrient availability (9). In intensive crops, such as lettuce and tomato, the recommended dose of an organic fertilizer aimed at maintaining soil productive capacity is 40 t ha^{-1} (42). The results obtained in this study would limit GP utilization as an organic fertilizer, but applications at the lowest dose could be used as mulching in tomatoes and possibly in other crops with similar tolerance ranges.

Conclusions

V. labrusca GP presents physicochemical characteristics associated with soil health. Its high content of phosphorus and potassium, as well as low sodium and low SAR values differentiate this winery by-product from the one derived from *V. vinifera*. Low concentrations of *V. labrusca* GP promotes *A. brasiliensis* and tomato root system without altering aerial biomass, an effect known as hormesis. Toxicity symptoms of *V. labrusca* GP on plant growth at highest doses restricts its usage as an organic fertilizer. However, further ongoing field experiments will evaluate the effect of GP as mulching on tomato growth and other microorganisms indicating soil biological quality.

References

1. Abbona, E. A.; Sarandón, S. J.; Marasas, M. E.; Astier, M 2007. Ecological sustainability evaluation of traditional management in different vineyard systems in Berisso, Argentina. Agriculture, ecosystems & environment. 119(3-4): 335-345. https://doi.org/10.1016/j.agee.2006.08.001
2. Alburquerque, J. A.; Gonzálvez, J.; García, D.; Cegarra, J. 2004. Agrochemical characterisation of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. Bioresour Technol. 91: 195-200. https://doi.org/10.1016/S0960-8524(03)00177-9
3. Boulton, R. 1980. The relationships between total acidity, titratable acidity and pH in wine. Am J Enol Vitic 31.
4. Braham, J. E.; Bressani, R. 1979. Coffee pulp: composition, technology and utilization. IDRC. Ottawa. ON. CA.
5. Bremner, J. M.; Mulvaney, S. C. 1982. Nitrogen Total, in: Page, A. L.; Miller, R. H.; Keeney, D. R. (Eds.), Methods of Soil Analysis: Chemical and Microbiological Properties. Soil Science Society of America, Madison (WI). p. 595-624.
6. Bustamante, M. A.; Moral, R.; Paredes, C.; Pérez-Espinosa, A.; Moreno-Caselles, J.; Pérez-Murcia, M. D. 2008. Agrochemical characterisation of the solid by-products and residues from the winery and distillery industry. Waste Manag. 28: 372-380.

7. Corbin, K. R.; Hsieh, Y. S. Y.; Betts, N. S.; Byrt, C. S.; Henderson, M.; Stork, J.; DeBolt, S.; Fincher, G. B.; Burton, R. A. 2015. Grape marc as a source of carbohydrates for bioethanol: Chemical composition, pre-treatment and saccharification. Bioresour Technol. 193: 76-83.
8. Devesa-Rey, R.; Vecino, X.; Varela-Alende, J. L.; Barral, M. T.; Cruz, J. M.; Moldes, A. B. 2011. Valorization of winery waste *vs*. the costs of not recycling. Waste Manage. 31: 2327-2335.
9. Diacono, M.; Montemurro, F. 2011. Long-term effects of organic amendments on soil fertility. Sustain Agr. 2: 761-786.
10. Diacono, M.; Montemurro, F. 2015. Effectiveness of Organic Wastes as Fertilizers and Amendments in Salt-Affected Soils. Agriculture. 5: 221-230.
11. FAO. 1998. ISSS-ISRIC-FAO. World reference base for soil resources. Acco Press, Leuven, Belgium.
12. Fernández Linares, L. C.; Rojas Avelizapa, N.G.; Roldán Carillo, T. G.; Ramírez Islas, M. E.; Zegarra Martinez, H. G.; Uribe Hernández, R.; Reyes ävila R. J.; Flores Hernández, D.; Arce Ortega, J. M. 2006. Manual de técnicas de análisis de suelos aplicadas a la remediación de sitios contaminados. Instituto Mexicano del Petróleo, México.
13. Fukami, J.; Cerezini, P.; Hungria, M. 2018. Azospirillum: benefits that go far beyond biological nitrogen fixation. AMB Express 2018(8): 1-12. https://doi.org/10.1186/S13568-018-0608-1
14. Funes-Pinter, I.; Salomón, M. V.; Martín, J. N.; Uliarte, E. M.; Hidalgo, A. 2022. Effect of bioslurries on tomato *Solanum lycopersicum* L and lettuce *Lactuca sativa* development. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(2): 48-60. DOI: https://doi.org/10.48162/rev.39.082
15. Garrido Valero, S. 1994. Interpretación de análisis de suelos, N° 5/93. Instituto Nacional de Reforma y Desarrollo Agrario. Madrid.
16. González Fernández, J. L.; Eraso Luca de Tena, F.; Pérez Lanzac, J.; Medina Blanco, M. 1991. Influencia del procesado y fecha de recogida sobre la composición química de los orujos de uva. Arch Zootec. 40: 379-389.
17. Havlin, J. L.; Beaton, J. D.; Tisdale, S. L.; Nelson, W. L. 1999. Soil fertility and fertilizers, 6th ed. Prentice Hall, Upper Saddle River. New York.
18. Hernández, J. D.; Trujillo, Y. Y.; Osorio, D. S. D. 2011. Phenolic potential determination and yeasts identification with significant leavens in Isabella grape (*Vitis labrusca*) from Villa del Rosario (Norte de Santander) for wine making. Vitae. 18: 17-25.
19. Jackson, M. L.; Barak, P 2005. Soil chemical analysis: advanced course: a manual of methods useful for instruction and research in soil chemistry, physical chemistry of soils, soil fertility, and soil genesis. Parallel Press. University of Wisconsin-Madison Libraries.
20. Kurt-Celebi, A.; Colak, N.; Hayirlioglu-Ayaz, S.; Veličkovska, S. K.; Ilieva F.; Esatbeyoglu, T.; Ayaz, F. A. 2020. Accumulation of phenolic compounds and antioxidant capacity during Berry development in Black 'Isabel' Grape (*Vitis vinifera* L. x *Vitis labrusca* L.). Molecules. 25: 3845-3845.
21. Mateo, J. J.; Maicas, S. 2015. Valorization of winery and oil mill wastes by microbial technologies. Int Food Res J. 73: 13-25. https://doi.org/10.1016/J.FOODRES.2015.03.007
22. Mazzarino, M. J.; Satti, P. 2012. Compostaje en la Argentina: experiencias de producción, calidad y uso. Editorial UNRN.
23. Möller, K.; Schulthei, U. 2015. Chemical characterization of commercial organic fertilizers. Arch Agron Soil Sci. 61: 989-1012.
24. Nascimento-Gavioli, M. C. A.; Rockenbach, M. F.; Welter, L.; Guerra, M. P. 2019. Histopathological study of resistant (*Vitis labrusca* L.) and susceptible (*Vitis vinifera* L.) cultivars of grapevine to the infection by downy mildew. J Hortic Sci Biotechnol. 95: 521-531. https://doi.org/10.1080/14620316.2019.1685411
25. Ngosong, C.; Okolle, J. N.; Tening, A. S. 2019. Mulching: A sustainable option to improve soil health. Soil Fertility Management for Sustainable Development. p. 231-249.
26. Obiakor, M. O.; Wilson, S. C.; Tighe, M.; Pereg, L. 2019. Antimony Causes Mortality and Induces Mutagenesis in the Soil Functional Bacterium *Azospirillum brasilense* Sp7. Water Air Soil Pollut 230 1–14. https://doi.org/10.1007/S11270-019-4232-8
27. OIV International Organization of Vine and Wine 2019 n.d. Statistical report on world vitiviniculture [WWW Document]. https://www.oiv.int/es/technical-standards-and-documents/statistical-analysis/annual-assessment (accessed 10.6.22).
28. Osono, T.; Takeda, H. 2001. Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. Ecol Res. 16: 649-670.
29. Otero, J. 2013. Factores de la reactivación de un producto agroalimentario típico: el vino de la costa de Berisso, Argentina. Cuadernos de Desarrollo Rural. 10(71): 37-58.
30. Ren, S. 2003. Phenol mechanism of toxic action classification and prediction: a decision tree approach. Toxicol Lett. 144: 313-323. https://doi.org/10.1016/S0378-4274(03)00236-4
31. Richards, L. A. 1954. Diagnosis and improvement of saline and alkali soils. Agriculture Handbook N° 60. Washington DC.

32. Rodríguez Larramendi, L. A.; Salas-Marina, M. Á.; Hernández García, V.; Campos Saldaña, R. A.; Cruz Macías, W.; López Sánchez, R. 2023. Seed treatments with salicylic acid and Azospirillum brasilense enhance growth and yield of maize plants (Zea mays L.) under field conditions. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(1): 17-26. DOI: https://doi.org/10.48162/rev.39.092
33. Romero-Romero, T.; Sánchez-Nieto, S.; San Juan-Badillo, A.; Anaya, A. L.; Cruz-Ortega, R 2005. Comparative effects of allelochemical and water stress in roots of *Lycopersicon esculentum* Mill. (Solanaceae). Plant Sci. 168: 1059-1066.
34. Rondeau, P.; Gambier, F.; Jolibert, F.; Brosse, N. 2013. Compositions and chemical variability of grape pomaces from French vineyard. Ind Crops Prod. 43: 251-254.
35. SAGPyA 2004. Sistema de apoyo metodológico a los laboratorios de suelos, agua, vegetales y enmiendas orgánicas (SAMLA)
36. Sampedro, I.; Aranda, E.; Martín, J.; García-Garrido, J. M.; García-Romera, I.; Ocampo, J. A. 2004. Saprobic fungi decrease plant toxicity caused by olive mill residues. Appl Soil Ecol. 26: 149-156. https://doi.org/10.1016/j.apsoil.2003.10.011
37. Saparrat, M. C. N.; Jurado, M.; Díaz, R.; Romera, I. G.; Martínez, M. J. 2010. Transformation of the water soluble fraction from “alpeorujo” by *Coriolopsis rigida*: The role of laccase in the process and its impact on *Azospirillum brasiliense* survival. Chemosphere. 78: 72-76. https://doi.org/10.1016/j.chemosphere.2009.09.050
38. Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 9: 671-675. https://doi.org/10.1038/nmeth.2089
39. Scotti, R.; Bonanomi, G.; Scelza, R.; Zoina, A.; Rao, M. A. 2015. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. J Soil Sci Plant Nutr. 15: 333-352.
40. Sisterna, M.; Ronco, L.; Voget, C.; Marasas, M.; Abbona, E.; Romero, M.; Daniele, J.; Artaza, S.; Otero, J.; Sepúlveda, C.; Avila, G.; Loviso, C.; Orosco, E.; Bonicatto, M.; Condes, C.; Velarde, I. 2010. American Grapevine Culture and Research in Berisso, Argentina. Am J Plant Sci. 3: 38-53.
41. Tiquia, S. M.; Tam, N. F. Y.; Hodgkiss, I. J. 1996. Effects of composting on phytotoxicity of spent pigmanure sawdust litter. Environ Pollut. 93: 249-256.
42. Vazquez, M. E.; Terminiello, A. 2012. Recuperación de suelos degradados de pequeños productores del cinturón hortícola del Gran La Plata. Valoración del problema y estrategias correctivas. FCAyF-UNLP.
43. Walkley, A.; Black, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-38. https://doi.org/10.1097/00010694-193401000-00003
44. Wang, X.; Sun, C.; Wang, Y.; Wang, L. 2002. Quantitative structure-activity relationships for the inhibition toxicity to root elongation of *Cucumis sativus* of selected phenols and interspecies correlation with *Tetrahymena pyriformis*. Chemosphere. 46: 153-161. https://doi.org/10.1016/S0045-6535(01)00133-3
45. Zaghloul, A.; Saber, M.; Gadow, S.; Awad, F. 2020. Biological indicators for pollution detection in terrestrial and aquatic ecosystems. Bulletin of the National Research Centre. 44(1): 1-11. https://doi.org/10.1186/s42269-020-00385-x

Acknowledgements

Special thanks to Dr. Mario C.N. Saparrat for his support during the development of the experiments and critical reading of the manuscript.

The authors are grateful for the financial support provided by Agencia Nacional de Promoción Científica y tecnológica (PICT 2019-00207 to Mario C.N. Saparrat; PICT 2021–00056 to María Inés Troncozo)

A polyphasic study of non-aflatoxigenic *Aspergillus flavus* Link, isolates from maize in the Chaco semi-arid region of Argentina

Estudio polifásico de aislados no aflatoxigénicos de *Aspergillus flavus* Link, en maices de la región del Chaco semiárido argentino

Javier Barontini [1, 2, 3, 4 *], María Silvina Alaniz Zanon [6], Ada Karina Torrico [5], Marcelo Druetta [7], Ignacio Martín Luna [7], Agustina Ruiz Posse [8], Sofía Noemí Chulze [6], María de la Paz Giménez Pecci [5]

Originales: *Recepción:* 02/08/2023 - *Aceptación:* 07/05/2024

Abstract

Maize (*Zea mays* L.) is one of the most widely planted crops globally with Argentina leading world production and exportation. Santiago del Estero province, east of Tucumán and north of Córdoba encompasses eight agro-climatic zones in the Chaco Semi-arid region, agro-ecologically characterized by a wide temperature range and frequent drought periods that expose the crop to pathogens, particularly *Aspergillus flavus*. This pathogen is responsible for ear rot and grain contamination with mycotoxins such as aflatoxin B_1 and cyclopiazonic acid. This study obtained fungal isolates from ears of maize and characterized them according to toxigenic capability and morphotype of sclerotia (S < 400 μm, associated with high levels of aflatoxins and L > 400 μm, related to variable levels of aflatoxins). In addition, those not producing aflatoxins were studied to determine phylogenetic relationships based on sequences of a segment of the *CaM* gene. Fifty-eight isolates were obtained in eight localities representing each agro-climatic zone, 30 of which were non-aflatoxigenic, 28 aflatoxigenic, and all producers of ciclopiazonic acid. Six isolates did not produce sclerotia, 51 were L and only one was S, the latter being a non-producer of aflatoxins. The number of sclerotia was positively correlated with the production of aflatoxin B_1, while size was negatively correlated. The *CaM* gene sequences corroborated that the isolates belonged to the *A. flavus* clade and the high nucleotide similarity among them (99.4% to 100%) revealed almost zero genetic diversity in this geographic region. No significant differences were observed in the proportion of isolates between growing seasons or among agroclimatic districts. This research revealed characteristics of fungus populations in this agricultural region of north Argentina.

Keywords

non-aflatoxigenic isolate • cyclopiazonic acid • sclerotia production • phylogenetic relationships

1 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Cátedra de Fitopatología. Almirante Brown 500. Chacras de Coria. Mendoza. Argentina. M5528AHB. * jbarontini@fca.uncu.edu.ar
2 Instituto Nacional de Tecnología Agropecuaria (INTA). Estación Experimental Mendoza. San Martín 3853. Luján de Cuyo. Mendoza. Argentina. 5507
3 Instituto de Biología Agrícola de Mendoza (IBAM). Almirante Brown 500. Chacras de Coria. Mendoza. Argentina. M5528AHB.
4 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Godoy Cruz 2290. Ciudad Autónoma de Buenos Aires. Argentina. C1425FQB.
5 INTA. Instituto de Patología Vegetal (IPAVE). Centro de Investigaciones Agropecuarias (CIAP). Av. 11 de Septiembre 4755. Córdoba. Córdoba. Argentina. X5014MGO.
6 Instituto de Investigación en Micología y Micotoxicología (IMICO). CONICET. Universidad Nacional de Río Cuarto (UNRC). Ruta Nac. 36 km 601. Río Cuarto. Córdoba. Argentina. X5804BYA.
7 Estación Experimental Agropecuaria. Este de Santiago del Estero. Instituto Nacional de Tecnología Agropecuaria (EEA - ESE - INTA). Ruta Prov. N° 6 Km 9. Quimilí. Santiago del Estero. Argentina. G3740CDA.
8 Unidad de Fitopatología y Modelización Agrícola (UFyMA). INTA. CONICET. Av. 11 de Septiembre 4755. Córdoba. Córdoba. Argentina. X5014MGO.

Resumen

El maíz (*Zea mays* L.) es uno de los cultivos más sembrados en el mundo, siendo Argentina líder mundial en producción y exportación. La Provincia de Santiago del Estero, el este de Tucumán y el norte de Córdoba abarcan ocho zonas agroclimáticas en la región Chaco semiárida, la cual se caracteriza por un amplio rango de temperatura y frecuentes sequías que exponen al cultivo a patógenos, particularmente *Aspergillus flavus,* responsable de la podredumbre de la espiga y la contaminación de granos con micotoxinas como aflatoxina B_1 y ácido ciclopiazónico. Se obtuvieron aislados del hongo desde espigas de maíz y se caracterizaron según capacidad toxigénica y morfotipos de esclerocios (S < 400 µm, asociados con elevados niveles de aflatoxinas y L > 400 µm, relacionados con niveles variables de aflatoxinas). Además, aquellos que no producían aflatoxinas se estudiaron para determinar sus relaciones filogenéticas sobre la base de un segmento del gen *CaM*. Cincuenta y ocho aislados fueron obtenidos en ocho localidades representativa de cada zona agroclimática, 30 de los cuales resultaron no aflatoxigénicos, 28 aflatoxigénicos y todos productores de ácido ciclopiazónico. Seis aislados no produjeron esclerocios, 51 fueron L y solo uno fue S, siendo este último no aflatoxigénico. El número de esclerocios correlacionó positivamente con la producción de aflatoxina B_1, mientras que el tamaño lo hizo de manera negativa. Las secuencias del gen *CaM* corroboraron que los aislados pertenecen al clado *A. flavus* y la alta similitud nucleotídica (99,4% a 100%) reveló casi nula diversidad genética en esta región. No se observaron diferencias en la proporción de aislados entre campañas agrícolas estudiadas ni distritos agroclimáticos. Esta investigación reveló características de las poblaciones de hongos en esta región agrícola del norte argentino.

Palabras clave

aislado no aflatoxigénico • ácido ciclopiazónico • producción de esclerocios • relaciones filogenéticas

Introduction

Argentina ranks sixth in maize production, with approximately 60 million tons a year. The region encompassing Santiago del Estero province, east of Tucumán and north of Córdoba, in the Chaco Semi-arid region, produces about 6 million tons (58). This geographical area is a critical climatic zone, where climatic factors are highly variable (57). Thus, maize crop development in these agroecosystems is constrained by stressful environmental factors that expose the crop to different pathogens, such as those responsible for ear rot (27, 67). The most dangerous of these pathogens is *Aspergillus flavus* Link, a globally distributed filamentous, cosmopolitan pathogen that causes opportunistic infections in animals and plants (12, 42). This pathogen is commonly present in air and soil mycobiota. Under certain water and thermal stress conditions, some isolates produce mycotoxins like aflatoxins and cyclopiazonic acid (CPA). Aflatoxins are secondary metabolites with strong hepatotoxic, teratogenic, immunosuppressive and mutagenic activity when inhaled, ingested or absorbed through animal and human skin (51); *A. flavus* is the main driver of aflatoxin contamination in maize in the world (45). While CPA produces liver necrosis, convulsions myocardium lesions in animals (62, 63), the specific exposure response to this toxin differs among species, without sufficient evidence about natural contamination of foods (81).

The maximum safety limit of aflatoxins for maize commercialization varies between countries, being 20 ng. g^{-1} in Argentina, Brazil and USA, 4 ng. g^{-1} in the European Union, and 15 ng. g^{-1} in African countries like Ghana (4, 9, 16, 34, 77).

Species of the genus *Aspergillus* produce sclerotia, resistance structures consisting of darkly pigmented and hardened hyphal mats (50) conferring different phenotypic characteristics (1). Thus, *Aspergillus* species can be classified into two morphotypes: S isolates, which produce numerous sclerotia under 400 µm in diameter, associated with high levels of aflatoxins, and L isolates, with fewer sclerotia, diameter over 400 µm, and related to variable levels of aflatoxins (75). A third type of isolate not producing sclerotia under laboratory conditions, has been identified in Argentina and Italy (13, 38).

The variability among *A. flavus* populations can be estimated by establishing phylogenetic relationships based on morphological and molecular markers (51), as in the case of the calmodulin gene (*CaM*) (36). Calmodulin (*CaM*) is a highly conserved polypeptide present in eukaryotic cells. In the genus *Aspergillus*, *CaM* is important in phosphorylation and dephosphorylation of proteins involved in the biosynthesis of aflatoxins (47).

Aflatoxin contamination usually starts in the field, during harvest of infected grains, becoming more severe during storage (35). In the field, spores of *A. flavus* are transported by biotic agents (insects, birds) and/or abiotic agents (wind, rain splash) to the maize ear. Spores enter through the silk during flowering and through the wounds of the ear cover or of developing kernels (66). Water stress, high temperatures, insect attack, certain sowing dates and high crop density favor contamination with the pathogen and the possible production of aflatoxins (48).

Some members of *A. flavus* were found unable to biosynthesize aflatoxins being, therefore, plausible biocontrol tools (23). Biological control applied in maize and peanut (6, 73), among other crops, is based on the competition for infection sites and essential nutrients between non-aflatoxigenic and aflatoxigenic isolates. Thus, non-aflatoxigenic isolates displace aflatoxigenic ones and reduce the latter's capacity to produce aflatoxins (70, 79).

Non-aflatoxin producing strains selected as biocontrol agents must meet some requirements, such as being native to the area of application, being able to displace the toxicogenic isolates in the ecosystem and thereby reduce infections (23) and be genetically incompatible with the remaining fungal population. Therefore, studies of the native fungal population are crucial (42).

This work aimed to study the diversity of *A. flavus* populations in the Chaco Semi-arid region of Argentina through the morphological, toxicological and molecular characterization of isolates obtained from maize ears.

Materials and methods

Sampling

Sampling was conducted during the 2015/16 and 2016/17 growing seasons in representative localities of eight agro-climatic zones: Sachayoj (Zone 4: Río Muerto), Quimilí (Zone 5: Hickmann), Bandera (Zone 17: Bandera), Santiago del Estero (Zone 18: Monteagudo), Sumampa (Zone 37: Soto), Santa Rosa de Leales (Zone 18A: Monteagudo), Rayo Cortado (Zone 37C: Soto-north of Córdoba) and Villa de Tulumba (Zone 61B: Deán Funes), located between -30.38 and -26.55 S and -61.82 and -65.27 W (25) (figure 1, page 61). Ten ears were randomly collected along a diagonal line in the plot, at plant physiological maturity before harvest. They were threshed to form a composite sample and oven-dried at 38°C for 72 hours until final humidity was under 12 %, therefore, minimizing fungus development (26).

Isolation and morphological and molecular identification

From each sample, 100 kernels were taken, surface-desinfested with a 1% sodium hypochlorite wash followed by three washes with distilled water and plated in DG-18 medium. Kernels were incubated for 7 days at 25°C and cultures with characteristics similar to those of *A. flavus* were transferred to MEA medium. They were incubated for 7 days at 25°C (65) and then identified through taxonomic keys (48). Spores of each isolate were serially diluted, and the most diluted concentration was cultured in 2 % Water - Agar (WA) medium and incubated for 18 hours at 27°C (65). The germinated conidia were identified under Nikon SMZ-10 stereoscopic microscope (15X). Two conidia were extracted from each cultured Petri dish, along with a portion of culture medium, and transferred to Petri dishes containing MEA medium. They were incubated for 7 days at 25°C. Then, one of them was stored to obtain the single spore cultures corresponding to each isolate.

Sampling localities / Localidades evaluadas: Sachayoj. Quimilí. Santiago del Estero. Bandera. Sumampa (Santiago del Estero province). Santa Rosa de Leales (Tucumán province). Rayo Cortado. Villa de Tulumba (Córdoba province).

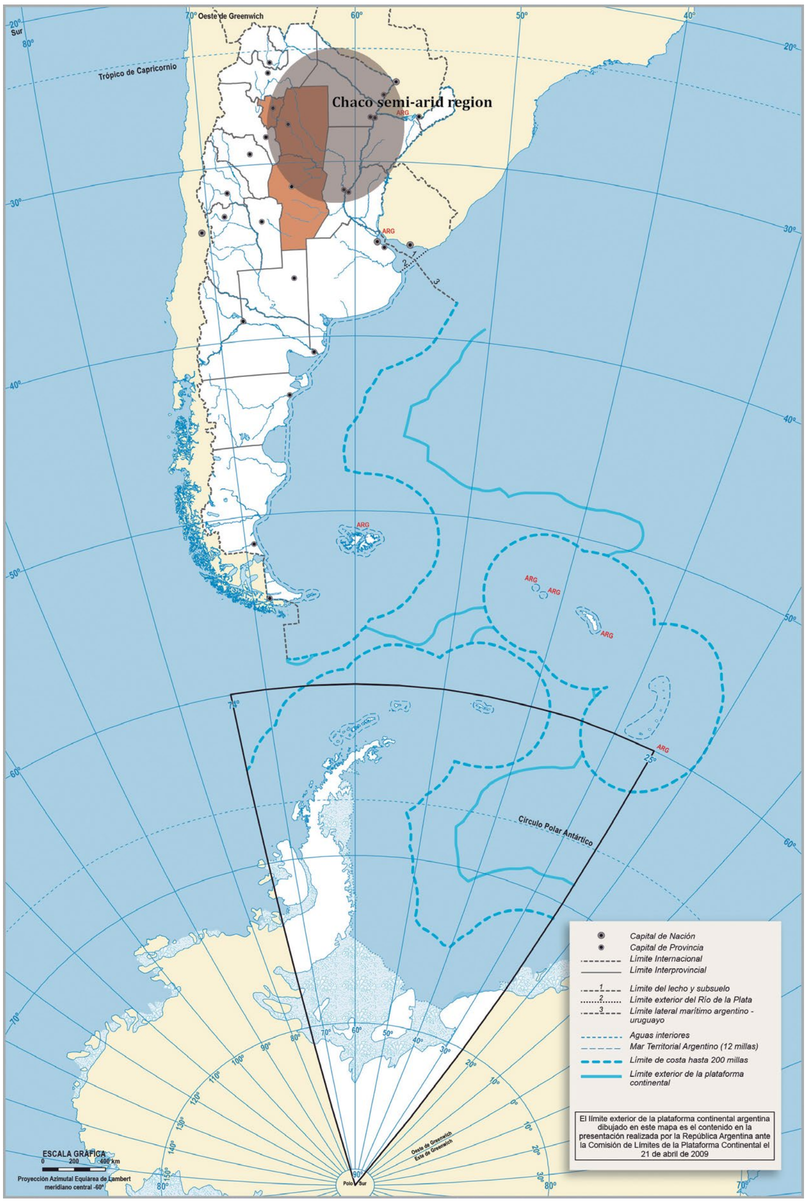


Figure 1. Sampling localities in the Chaco Semi-arid region of Argentina.

Figura 1. Localidades evaluadas en la región del Chaco semiárido argentino.

The identity of *A. flavus* was confirmed by PCR using the primers FLA1, 5'-GTAGG-GTTCCTAGCGAGCC-3'; FLA2 5'-GGAAAAAGATTGATTTGCGTTC-3' (41), with the ITS region as target, using the isolate *A. flavus* CCC 101-92 < NRRL 3251 as positive control, and the isolate *A. parasiticus* NRRL 2999 as negative control. Each single-spore isolate was cultured in MEA and incubated for 7 days at 25°C. Then, an aliquot of mycelium was taken, and DNA was extracted (55). For each reaction, 1 μL (final concentration of 20-100 ng of DNA.μL^{-1}, quantified in Nano Drop, Thermo Fisher Scientific, USA) of DNA solution was mixed with 24 μL of a solution composed of 5 μL 5x Green GoTaq® reaction buffer (Promega®, Madison, WI, USA), 0.5 μL of a mixture of dNTP (10 mM of dATP, dCTP, dGTP and dTTP), 1 μL of the primers (20 μM), 1 μL of polymerase enzyme GoTaq® (5U.μL^{-1}, Promega®, Madison, WI, USA) and 16.5 μL DEPC-treated water. The PCR reaction was performed following González Salgado *et al*. (2011). PCR products were revealed by agarose gel electrophoresis, visualized in UV transilluminator, with previous staining in GelRed™ Biotium solution (2.5 ng. μL^{-1}) and confirmed by the expected band size of 500 pb. Molecular size of the DNA fragments was estimated using the "qLadder 100 pb precision" marker (PB-L®, Bs As, Argentina).

Sclerotia production

Each isolate was cultured in 6-cm Petri dishes containing Czapeck dox (Cz) medium. They were inoculated in the center of the dish with 10 µL of a spore suspension and incubated for 14 days at 30°C (64). Sclerotia were recovered, their diameters measured under a microscope (Nikon eclipse Cs1 spectral) and characterized following Cotty (1989) into L or S morphotypes or isolates not producing (NP) sclerotia (60). Isolates not producing sclerotia after an incubation period were cultured in 5/2 medium for 5 to 7 days at 31°C to induce their production (38).

Toxigenic capability

Considering Aflatoxin B_1 (AFB_1) was one of the greatest frequency and toxicity of the four most important aflatoxins identified, its production was quantified (23, 82). *A. flavus* isolates were inoculated in duplicate on MEA slants for 7 days at 28°C. These cultures were used to prepare spore suspensions, following the method described by Alaniz Zanon *et al*. (2013). Spore concentration was measured in a Neubauer chamber and adjusted to 10^5 spores. mL^{-1}. Four-milliliter vials containing 1 mL broth from a medium containing 150 g sucrose, 20 g yeast extract, 10 g soytone, and 1 L distilled water, were inoculated with 100 µL of each spore suspension. Medium pH was adjusted to 5.9 with HCl. Cultures were incubated for 7 days at 30°C. Vial cultures were extracted according to Horn *et al.* (1996), by adding 1 mL of chloroform to each vial and vortexing for 30 s.

The first group of dried extracts was evaluated in their capacity to produce aflatoxins by HPLC according to Horn *et al.* (1996). Aflatoxins were analyzed by injecting 50 µL of extract from each vial into an HPLC system consisting of a Hewlett Packard model 1100 pump (Palo Alto, CA) connected to a Hewlett Packard model 1046A programmable fluorescence detector and a data module Hewlett Packard Kayak XA (HP ChemStation Rev.A.06.01). Chromatographic separations were performed on a stainless steel, C18 reversed-phase column (150 mm × 4.6 mm i.d., 5 µm particle size; Luna Phenomenex, Torrance, CA, USA) connected to a precolumn Security Guard (20 mm × 4.6 mm i.d., 5 µm particle sizes, Phenomenex). The mobile phase was water:methanol:acetonitrile (4:1:1, v/v/v) at a flow rate of 1.5 mL. min^{-1}. Pure aflatoxin solutions were used as external standards (Sigma-Aldrich, St. Louis, MO, USA).

The second group of dried extracts was resuspended in 500 µL of mobile phase of acetonitrile: $ZnSO_4$ 4 mM buffer solution (65:35, V/V) and CPA was determined in the HPLC system. Chromatographic separations were performed in Agilent ZORBAX RX-SIL column (250 mm x 4.6 mm i.d., 5 µm particle size) connected to a pre-column Security Guard (20 mm x 4.6 mm i.d., 5 µm particle sizes, Phenomenex) and 50 µl of each sample were analyzed at a flow velocity of 0.8 mL. min^{-1}.

The quantitative analysis was performed by normalization of peak areas. A calibration curve was elaborated from the areas obtained for the different concentrations of standards of aflatoxins and CPA, as applicable (Sigma Aldrich, St. Louis, MO, USA). The AFB_1 and CPA detection limit was 1 ng. g^{-1} (24, 32).

Sequencing and Phylogenetic relationships

Molecular variability of the non-aflatoxigenic isolates was explored using a 688-pb fragment of the *CaM* gene. We studied only this type of isolate given their potential for biocontrol strategies. Spores of the isolates cultivated in MEA medium were suspended for 7 days at 25°C. Conidia ($1x10^6$ conidia. mL^{-1}) were inoculated in 100 mL of lixiviated medium of potato glucose agar (10% of potato infusion from 200 g potato, 2% glucose, 4.5 pH). They were incubated in orbital shaker at 150 rpm for 48 hours at 25°C. Mycelium was collected by filtration and powdered with liquid N_2 (28). This culture medium allows one to obtain enough amount of mycelium. DNA was extracted using the cetyl-trimethylammonium bromide (CTAB) method (54), quantified by spectrophotometry and a segment of the *CaM* was amplified with the primers CL1, 5'- GA(GA)T(AT)CAAGGAGGCCTTCTC -3'; CL2A, 5'- TTTTTGCATCATGAGTTGGAC -3' (61). The obtained fragments of the expected size (688 pb) were purified using DNA columns of Wizard® SV Gel columns and PCR clean - Up system (Promega, Madison, WI, USA), following manufacturer instructions. Then, they were quantified by spectrophotometry. Both DNA chains were sent for sequencing by Sanger method (Macrogen, Seoul, South Korea). The obtained sequences were aligned using the software Clustal X2 (54). The software MEGA version 7 (53) was used to select the best nucleotide substitution model and to obtain the phylogenetic trees using Neighbor-Joining (NJ) (74) and Maximum-Likelihood (ML) methods (39, 76), both with 10,000 replicates bootstrap. The sequences were compared with the reference NW_002477238.1 and *A. niger* MH645004.1 was used as outgroup, both from the GenBank.

Statistical analysis

Data on sclerotia morphotypes, toxigenic capacity and variability of the sequences of the *CaM* genes were analyzed using InfoStat statistical software as well as correlation analyses (29). Mean values were obtained using analysis of variance (ANOVA) and differences between means were compared using Fisher's LSD test (P< 0.05).

Results and discussion

Morphological and molecular identification

A total of 58 isolates were obtained in the sampling localities from the eight agro-climatic zones (table 1, page 63-64) with *A. flavus* morphological characteristics, according to Klich (2002) (figure 2, page 64). *A. flavus* identity was confirmed using the specific primers FLA1 and FLA2 through amplification of a 500-pb band (41) (figure 3, page 65). The presence of isolates in the eight zones, each one being considered a different agroecological environment given different meteorological scenarios at each growing season shows the broad distribution of the fungus in the study region, as reported for other regions of the country (8, 17) and the world (11, 30, 32).

Table 1. Isolates of *Aspergillus flavus* from maize ears collected from representative localities of eight agro-climatic zones of the Chaco Semi-arid region of Argentina. Production of aflatoxin B_1, cyclopiazonic acid and sclerotia morphotype during the 2015/16 and 2016/17 growing seasons.

Tabla 1. Aislados de *Aspergillus flavus* de espigas de maíz colectadas en localidades representativas de ocho zonas agroclimáticas del Chaco semiárido argentino. Producción de aflatoxina B_1, ácido ciclopiazónico y morfotipo de esclerocios durante las campañas agrícolas 2015/16 y 2016/17.

Agro-climatic zones: 4: Río Muerto, 5: Hickmann, 17: Bandera, 18 and 18A: Monteagudo, 37: Soto, 37C: Soto - North of Córdoba, 61B: Dean Funes.

L: sclerotia of > 400 μm; S: sclerotia of < 400 μm; NP: non-producer of sclerotia.

AFB1: Aflatoxin B; CPA: cyclopiazonic acid.

Zonas agroclimáticas: 4: Río Muerto, 5: Hickmann, 17: Bandera, 18 y 18A: Monteagudo, 37: Soto, 37C: Soto - Norte de Córdoba, 61B: Deán Funes.

L: esclerocios de tamaño > 400 μm; S: esclerocios de tamaño < 400 μm; NP: no productor de esclerocios.

AFB_1: Aflatoxina B; CPA: ácido ciclopiazónico.

Isolate	Agro-climatic Zone	Locality	Sampling year	Sclerotia Morphotype	AFB_1 (ng. g^{-1})	CPA (ng. g^{-1})
ASRC1	37C	Rayo Cortado	2017	L	6.4	3.3
ASRC2	37C	Rayo Cortado	2017	NP	0.0	6.2
ASRC3	37C	Rayo Cortado	2017	NP	0.0	0.6
ASVT1	61B	Tulumba	2017	L	278.4	0.3
ASVT2	61B	Tulumba	2017	L	0.0	2.8
ASVT3	61B	Tulumba	2017	L	405.1	8.6
ASVT4	61B	Tulumba	2017	L	60.6	2.3
ASBA12	17	Bandera	2016	L	16.3	9.9
ASBA3	17	Bandera	2016	L	0.0	2.8
ASBA4	17	Bandera	2016	L	7.2	9.2
ASBA8	17	Bandera	2016	L	0.0	4.7
ASBA1	17	Bandera	2017	L	0.0	2.3
ASBA10	17	Bandera	2017	L	5.9	9.3
ASBA11	17	Bandera	2017	L	0.0	2.3
ASBA2	17	Bandera	2017	L	0.0	2.3
ASBA5	17	Bandera	2017	L	0.0	6.7
ASBA6	17	Bandera	2017	L	90.7	2.9
ASBA7	17	Bandera	2017	L	6.7	5.4
ASBA9	17	Bandera	2017	NP	0.0	6.9

Isolate	Agro-climatic Zone	Locality	Sampling year	Sclerotia Morphotype	AFB_1 (ng. g^{-1})	CPA (ng. g^{-1})
ASQU1	5	Quimilí	2016	L	7.2	5.6
ASQU10	5	Quimilí	2016	L	0.0	54
ASQU11	5	Quimilí	2016	L	2521.5	7.5
ASQU12	5	Quimilí	2016	L	17.6	5.8
ASQU13	5	Quimilí	2016	L	5.6	1.2
ASQU14	5	Quimilí	2016	L	5.6	8.6
ASQU15	5	Quimilí	2016	L	0.0	3.2
ASQU16	5	Quimilí	2016	L	0.0	4.2
ASQU17	5	Quimilí	2016	L	0.0	8.7
ASQU18	5	Quimilí	2016	L	0.0	8.7
ASQU19	5	Quimilí	2016	L	0.0	0.6
ASQU2	5	Quimilí	2016	L	0.0	0.6
ASQU20	5	Quimilí	2016	L	0.0	7.1
ASQU21	5	Quimilí	2016	L	499.1	5.9
ASQU3	5	Quimilí	2016	L	34	0.4
ASQU4	5	Quimilí	2016	L	9.3	8.1
ASQU5	5	Quimilí	2016	L	6.3	16.4
ASQU6	5	Quimilí	2016	L	6	6.2
ASQU8	5	Quimilí	2016	L	5.7	6.9
ASQU9	5	Quimilí	2016	L	0.0	8.6
ASQU7	5	Quimilí	2017	L	0.0	1.6
ASSA1	4	Sachayoj	2016	L	9.8	8.5
ASSA3	4	Sachayoj	2016	L	13.2	9.9
ASSA4	4	Sachayoj	2016	L	0.0	7.6
ASSA6	4	Sachayoj	2016	L	9.1	9.1
ASSA2	4	Sachayoj	2017	L	70.1	7.9
ASSA5	4	Sachayoj	2017	L	0.0	3.8
ASSE5	18	Sgo del Estero	2016	L	5.8	8.9
ASSE1	18	Sgo del Estero	2017	S	0.0	1.8
ASSE2	18	Sgo del Estero	2017	L	0.0	0.6
ASSE3	18	Sgo del Estero	2017	L	48958.1	2.2
ASSE4	18	Sgo del Estero	2017	L	290.9	4.9
ASSE6	18	Sgo del Estero	2017	NP	0.0	4.9
ASSE7	18	Sgo del Estero	2017	NP	0.0	0.6
ASSE8	18	Sgo del Estero	2017	L	0.0	2.0
ASSE9	18	Sgo del Estero	2017	L	0.0	0.8
ASSU1	37	Sumampa	2017	NP	0.0	2.9
ASSU2	37	Sumampa	2017	L	12.3	5.1
ASLE1	18A	Leales	2017	L	0.0	1.3

Agro-climatic zones: 4: Río Muerto, 5: Hickmann, 17: Bandera, 18 and 18A: Monteagudo, 37: Soto, 37C: Soto - North of Córdoba, 61B: Dean Funes.

L: sclerotia of > 400 μm; S: sclerotia of < 400 μm; NP: non-producer of sclerotia.

AFB1: Aflatoxin B; CPA: cyclopiazonic acid.

Zonas agroclimáticas: 4: Río Muerto, 5: Hickmann, 17: Bandera, 18 y 18A: Monteagudo, 37: Soto, 37C: Soto - Norte de Córdoba, 61B: Deán Funes.

L: esclerocios de tamaño > 400 μm; S: esclerocios de tamaño < 400 μm; NP: no productor de esclerocios.

AFB_1: Aflatoxina B; CPA: ácido ciclopiazónico.

Figure 2. *Aspergillus flavus* Link colony on malt extract agar (MEA) culture medium.
Figura 2. Colonia de *Aspergillus flavus* Link en medio de cultivo agar extracto de malta (MEA).

M: molecular marker (100-pb DNA Ladder); +: *Aspergillus flavus* NRRL 3251 (positive control); -: *A. parasiticus* NRRL2999 (negative control); 1: ASSA2; 2: ASQU7; 3: ASQU21; 4: ASQU10; 5: ASQU15; 6: ASRC2; 7: ASQU12; 8: ASQU6; 9: ASBA3; 10: ASVT1.

M: marcador molecular (100-pb DNA Ladder); +: *Aspergillus flavus* NRRL 3251 (control positivo); -: *A. parasiticus* NRRL2999 (control negativo); 1: ASSA2; 2: ASQU7; 3: ASQU21; 4: ASQU10; 5: ASQU15; 6: ASRC2; 7: ASQU12; 8: ASQU6; 9: ASBA3; 10: ASVT1.

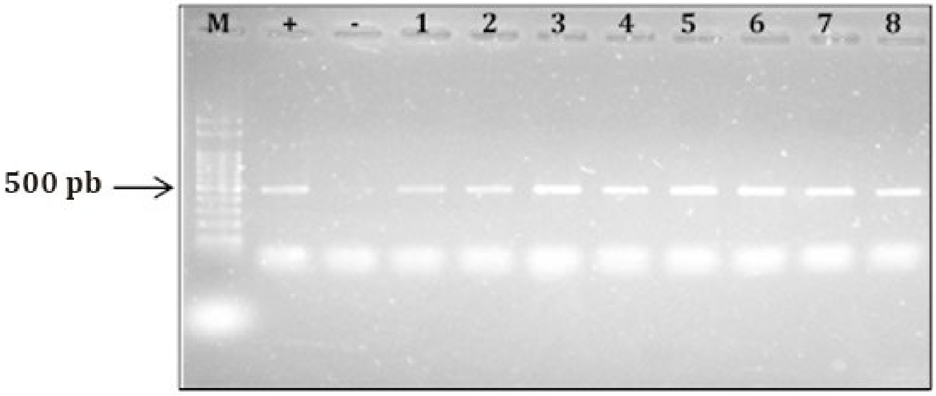


Figure 3. Electrophoresis gel showing PRC products obtained using the specific primers FLA1 and FLA2 (González Salgado *et al.*, 2011).

Figura 3. Gel de electroforesis mostrando productos de PCR obtenidos utilizando los iniciadores específicos FLA1 y FLA2 (González Salgado *et al.*, 2011).

Sclerotia production

Of the 58 *A. flavus* isolates identified, 6 (10%) produced no sclerotia, whereas 52 (90%) produced sclerotia. Of the latter, 51 (98%) were identified as L morphotype (figure 4), with > 400 µm in diameter, and only isolate, ASSE1, (2%) corresponded to S morphotype, with < 400 µm in diameter (table 1, page 63-64). Similar proportions between L, S and NP morphotypes in maize kernels were reported by Moreno (2004) in Mexico and by Pildain *et al.* (2005) in Argentina in peanut isolates. The low or null presence of S morphotype in maize was also reported by Giorni *et al*. (2007) in Italy. These authors identified one S isolate out of 70 isolates, with the remaining ones being NP. Similarly, Alaniz Zanon *et al*. (2018) reported a high proportion of L morphotype in Argentina with respect to NP, with absent S morphotype.

Sclerotia production was reported in isolates from all the agro-climatic zones and in both evaluated growing seasons. Dominance of L morphotype among isolates obtained in the Chaco Semi-arid region agrees with previous records in Argentina (7, 18) and in other countries, like Brazil, Portugal, Nigeria and Sub-Saharan Africa regions (5, 20, 33, 68, 72).

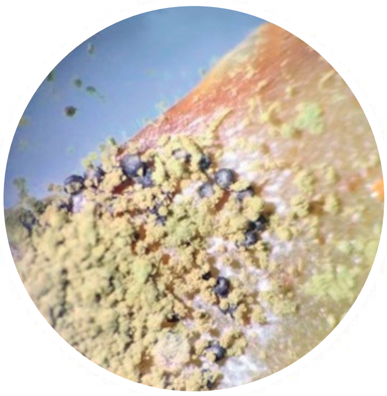


Figure 4. Sclerotia L morphotype in aflatoxigenic isolate of *Aspergillus flavus.* Observation under stereo microscope Nikon SMZ-10 (15X).

Figura 4. Esclerocios morfotipo L en aislado aflatoxigénico de *Aspergillus flavus.* Observación bajo lupa estereoscópica Nikon SMZ-10 (15X).

The high incidence of S morphotype is associated with regions of low precipitation and high temperatures, where high number of small sclerotia can be a survival trait facing rapid temperature and humidity fluctuations (19). However, and even though the study region is dry and hot, this was not observed.

Toxigenic capability

Based on the production of AFB_1 and CPA of the 58 isolates obtained, we identified 30 (52%) non-aflatoxigenic and 28 (48%) aflatoxigenic. This similarity of proportions was indicated by Martins *et al.* (2017) in peanut crop in Brazil and Camiletti *et al.* (2018) in maize ears in Argentina.

Aflatoxin-producer and non-producer isolates were obtained in all the agro-climatic zones and growing seasons, except Santa Rosa de Leales, where the only isolate found was non-producer.

Of the 28 isolates producing AFB_1, 26 (93%) had concentrations below 500 ng. g^{-1}, whereas ASQU11 and ASSE3 were higher aflatoxin producers of 2,521.5 ng. g^{-1} and 48,958.1 ng. g^{-1} respectively (table 1, page 63-64). Although the capacity to produce mycotoxins may depend on geographical origin and environmental conditions (62), our results do not show any pattern associated with zones or growing seasons. This agrees with Bayman and Cotty (1993), who did not detect differential patterns between nearby zones.

All isolates were CPA producers, indicating toxicity importance (78) and plausible use as aflatoxin biocontrol strategies. This is the case of the AF36 agent used in competitive exclusion strategies and found responsible for CPA increase in maize kernels inoculated in the field, and peanuts (2, 31). By contrast, Camiletti *et al*. (2018) detected 19% of CPA non-producer isolates in maize kernels in Argentina. On the other hand, Vaamonde *et al*. (2003) studied wheat, soybean and peanut in Argentina and found between 6% and 27% of isolates not producing this mycotoxin. In Italy, Giorni *et al*. (2007) reported 39% CPA non-producer isolates in maize kernels, whereas, in organic nut plantations in Brazil, Reis *et al*. (2014) found that 34% of the *A. flavus* isolates were non-producers of that mycotoxin. In addition, Jamali *et al*. (2012) identified 19 % of the isolates as CPA non-producers in soil samples of pistachio plantations in Czech Republic.

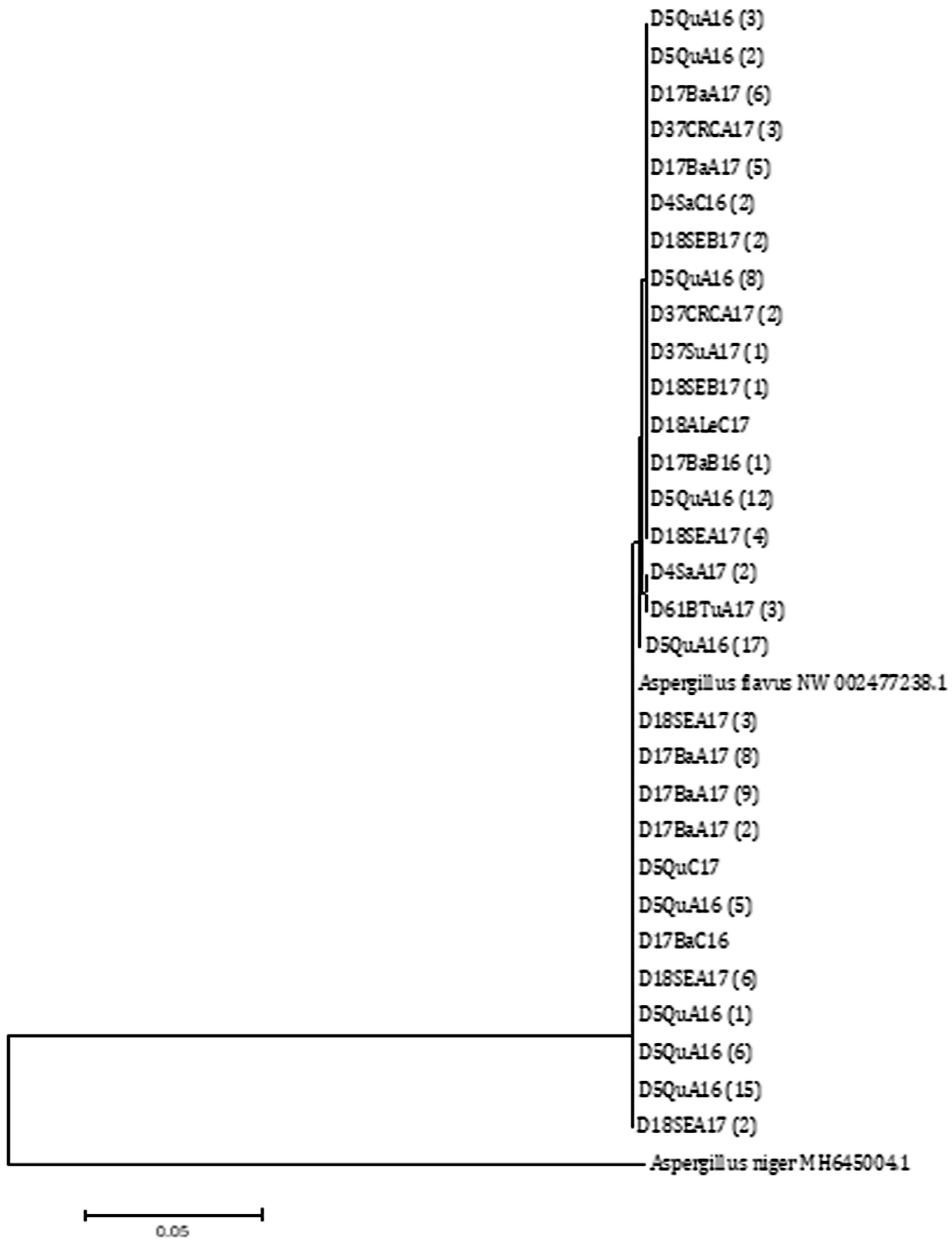
The presence of isolates producing both CPA and aflatoxins was reported in Argentina and other parts of the world (8, 18, 37, 71). The isolates with the highest AFB_1 production obtained in the Chaco Semi-arid region do not correspond to those with the highest CPA production.

Correlations between AFB_1 production and the number (r= 062) and size of sclerotia (r= -0.38) indicate that the produced amount of AFB_1 increases as sclerotia size decreases. These results are similar to those reported by Arrúa Alvarenga *et al*. (2012) and Pildain *et al*. (2005), but differ from Bouti *et al*. (2020), who did not find any correlation between sclerotia and aflatoxin production. No correlation was observed between CPA production and sclerotia morphotype.

The only S morphotype identified in this study (ASSE1), collected in 2016/17 growing season, did not produce AFB_1. While the presence of non-aflatoxin-producing isolates was also reported for USA, Ghana and Brazil (3, 22, 40, 44), in general, S morphotype isolates produce higher aflatoxin concentrations than L or NP morphotypes and are identified as major causal agents of severe contamination in maize and of most human deaths due to aflatoxicosis (13, 23). The S isolates are worldwide distributed, associated with aflatoxins B production in the USA and Africa (68), and of both B and G aflatoxins (3, 38).

Sequencing and phylogenetic analysis

All the *CaM* gene sequences studied in this work, using both NJ and ML, belong to the *A. flavus* clade (figure 5, page 67 and figure 6, page 68). The high similarity among isolates for that character showed limited genetic diversity in this geographic region. In addition, the only S morphotype is located together with L morphotype isolates, all of which are aflatoxin non-producers. This finding may be attributed to the fact that these are young populations and, therefore, may have not undergone sufficient mutations or recombination events leading to variability among isolates from the region (42, 62). However, only the northern zones of the region (4 and 5) correspond to land recently converted to agriculture, whereas in the remaining districts, both commercial hybrids and maize for self-consumption have been cultivated for several decades.

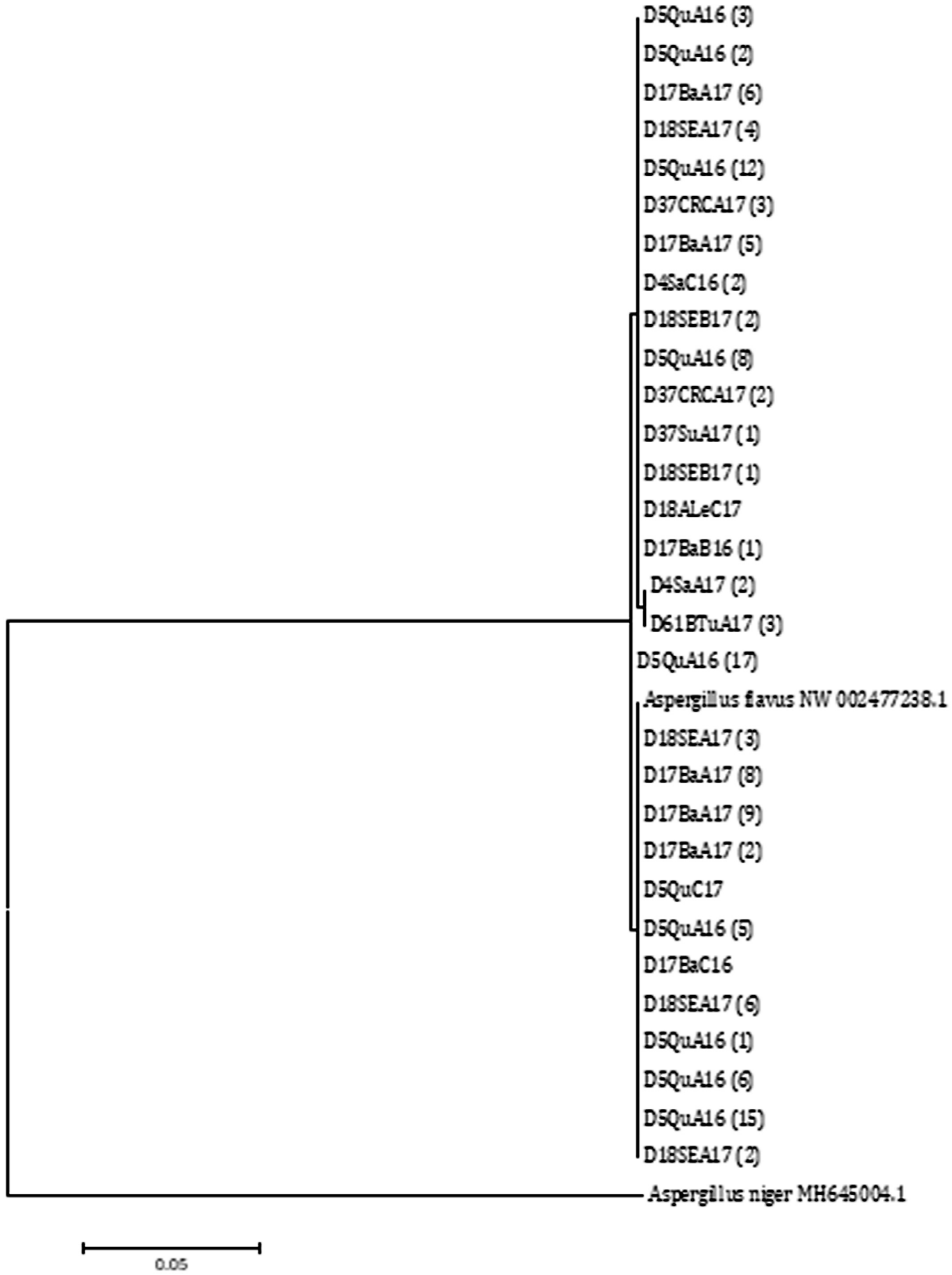


Node number corresponds to bootstrap support values (10000 replicates). *A. flavus* NW 002477238.1 was selected as reference sequence and *A. niger* MH645004.1 as outgroup sequence.

El número de nodos corresponde a un valor de bootstrap de 10000 réplicas. *A. flavus* NW 002477238.1 se utilizó como secuencia de referencia y *A. niger* MH645004.1 como secuencia externa al grupo.

Figure 5. Phylogenetic tree built using the Neighbor-Joining (NJ) statistic method based on the relationship among segments of the sequence of the *CaM* gene from *A. flavus* isolates from agro-climatic zones of the Chaco Semi-arid region in Argentina.

Figura 5. Árbol filogenético realizado con el método estadístico Neighbor-Joining (NJ) basado en las relaciones entre las secuencias de segmentos del gen *CaM* de aislados de *A. flavus* provenientes de zonas agroclimáticas del Chaco semiárido argentino.



Node number corresponds to bootstrap support values (10000 replicates). *A. flavus* NW 002477238.1 was selected as reference sequence and *A. niger* MH645004.1 as outgroup sequence.

El número de nodos corresponde a un valor de bootstrap de 10000 réplicas. *A. flavus* NW 002477238.1 se utilizó como secuencia de referencia y *A. niger* MH645004.1 como secuencia externa al grupo.

Figure 6. Phylogenetic tree built using the Maximum-Likelihood (ML) statistical method based on the relationship among segments of sequences of the *CaM* gene from *A. flavus* isolates from agro-climatic zones in the Chaco Semi-arid region in Argentina.

Figura 6. Árbol filogenético realizado con el método estadístico Maximum-Likelihood (ML) basado en las relaciones entre las secuencias de segmentos del gen *CaM* de aislados de *A. flavus* provenientes de zonas agroclimáticas del Chaco semiárido argentino.

Conclusions

A. flavus is present in all localities of the sampled agro-climatic zones and both growing seasons, evidencing the wide distribution of the pathogen in the Chaco Semi-arid region.

The population of *A. flavus* exhibits diversity in terms of sclerotia morphotypes and production of AFB_1 and CPA.

Low genetic variability among the isolates was observed, all belonging to the *A. flavus* clade.

In the future, genes from more variable regions will be used to perform phylogenetic analyses among *A. flavus* isolates.

References

1. Abbas, H. K.; Weaver, M. A.; Zablotowicz, R. M.; Horn, B. W.; Shier, W. T. 2005. Relationships between aflatoxin production and sclerotia formation among isolates of *Aspergillus* section Flavi from the Mississippi Delta. European Journal of Plant Pathology. 112(3): 283-287. https://doi.org/10.1007/s10658-004-4888-8
2. Abbas, H. K.; Weaver, M. A.; Horn, B. W.; Carbone, I.; Monacell, J. T.; Shier, W. T. 2011. Selection of *Aspergillus flavus* isolates for biological control of aflatoxins in corn. Toxin Reviews. 30(2-3): 59-70. https://doi.org/10.3109/15569543.2011.591539
3. Agbetiameh, D.; Ortega Beltran, A.; Awuah, R. T.; Atehnkeng, J.; Cotty, P. J.; Bandyopadhyay, R. 2018. Prevalence of aflatoxin contamination in maize and groundnut in Ghana: Population structure, distribution, and toxigenicity of the causal agents. Plant Disease. 102(4): 764-772. https://doi.org/10.1094/PDIS-05-17-0749-RE
4. Agbetiameh, D.; Ortega Beltran, A.; Awuah, R. T.; Atehnkeng, J.; Islam, M. S.; Callicott, K. A.; Cotty, P. J.; Bandyopadhyay, R. 2019. Potential of atoxigenic *Aspergillus flavus* vegetative compatibility groups associated with maize and groundnut in Ghana as biocontrol agents for aflatoxin management. Frontiers in Microbiology. 10: 1-15. https://doi.org/10.3389/fmicb.2019.02069
5. Ait Mimoune, N.; Arroyo Manzanares, N.; Gámiz Gracia, L.; García Campaña, A. M.; Bouti, K.; Sabaou, N.; Riba, A. 2018. *Aspergillus* section Flavi and aflatoxins in dried figs and nuts in Algeria. Food Additives & Contaminants: Part B. 11(2): 119-125. https://doi.org/10.1080/19393210.2018.1438524
6. Alaniz Zanon, M. S.; Chiotta, M.; Giaj Merlera, G.; Barros, G.; Chulze, S. 2013. Evaluation of potential biocontrol agent for aflatoxin in Argentinean peanuts. International Journal of Food Microbiology. 162(3): 220-225. https://doi.org/10.1016/j.ijfoodmicro.2013.01.017
7. Alaniz Zanon, M. S.; Barros, G. G.; Chulze, S. N. 2016. Non-aflatoxigenic *Aspergillus flavus* as potential biocontrol agents to reduce aflatoxin contamination in peanuts harvested in Northern Argentina. International Journal of Food Microbiology. 231: 63-68. https://doi.org/10.1016/j.ijfoodmicro.2016.05.016
8. Alaniz Zanon, M. S.; Clemente, M. P.; Chulze, S. N. 2018. Characterization and competitive ability of non-aflatoxigenic *Aspergillus flavus* isolated from the maize agro-ecosystem in Argentina as potential aflatoxin biocontrol agents. International Journal of Food Microbiology. 277(March). 58-63. https://doi.org/10.1016/j.ijfoodmicro.2018.04.020
9. ANVISA. Agencia Nacional de Vigilancia Sanitaria. 2011. Resolución RDC N° 7. Disposición sobre límites máximos tolerados para micotosinas en alimentos. Diario oficial de la Unión, Poder Ejecutivo, Brasilia. DF. 1: 66-67.
10. Arrúa Alvarenga, A. A.; Moreno Martínez, E.; Quezada Viay, M. Y.; Moreno Lara, J.; Vázquez Badillo, E.; Flores Olivas, A. 2012. *Aspergillus* aflatoxigénicos: enfoque taxonómico actual. Revista Mexicana de Ciencias Agrícolas. 3(4): 1047-1052. https://doi.org/10.29312/remexca.v3i5.1414
11. Bandyopadhyay, R.; Ortega Beltran, A.; Akande, A.; Mutegi, C.; Atehnkeng, J.; Kaptoge, L.; Senghor, A. L.; Adhikari, B. N.; Cotty, P. J. 2016. Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. World Mycotoxin Journal. 9(5): 771-789. https://doi.org/10.3920/WMJ2016.2130
12. Baranyi, N.; Kocsubé, S.; Jakšić Despot, D.; Šegvić Klarić, M.; Szekeres, A.; Bencsik, O.; Kecskeméti, A.; Manikandan, P.; Tóth, B.; Kredics, L.; Khaled, J. M.; Alharbi, N. S.; Vágvölgyi, C.; Varga, J. 2017. Combined genotyping strategy reveals structural differences between *Aspergillus flavus* lineages from different habitats impacting human health. Journal of Basic Microbiology. 57(11): 899-909. https://doi.org/10.1002/jobm.201700243
13. Barros, G. G.; Torres, A.; Rodriguez, M.; Chulze, S. 2006. Genetic diversity within *Aspergillus flavus* strains isolated from peanut-cropped soils in Argentina. Soil Biology and Biochemistry. 38(1): 145-152. https://doi.org/10.1016/j.soilbio.2005.04.028
14. Bayman, P.; Cotty, P. J. 1993. Genetic diversity in *Aspergillus flavus*: association with aflatoxin production and morphology. Canadian Journal of Botany. 71(1): 23-31. https://doi.org/10.1139/b93-003

15. Bouti, K.; Verheecke Vaessen, C.; Mokrane, S.; Meklat, A.; Djemouai, N.; Sabaou, N.; Mathieu, F.; Riba, A. 2020. Polyphasic characterization of *Aspergillus* section Flavi isolated from animal feeds in Algeria. Journal of Food Safety. 40(1): 1-9. https://doi.org/10.1111/jfs.12743
16. CAA. Código Alimentario Argentino. 2019. Secretaría de Alimentos, Bieoconomía y Desarrollo Regional Resolución Conjunta 22/ 2019 and 9/2021.
17. Camiletti, B. X.; Torrico, A. K.; Maurino, M. F.; Cristos, D.; Magnoli, C.; Lucini, E. I.; Giménez Pecci, M. P. 2017. Fungal screening and aflatoxin production by *Aspergillus* section Flavi isolated from pre-harvest maize ears grown in two Argentine regions. Crop Protection. 92: 41-48. https://doi.org/10.1016/j.cropro.2016.10.012
18. Camiletti, B. X.; Moral, J.; Asensio, C. M.; Torrico, A. K.; Lucini, E. I.; Giménez Pecci, M. P.; Michailides, T. J. 2018. Characterization of argentinian endemic *Aspergillus flavus* isolates and their potential use as biocontrol agents for mycotoxins in maize. Phytopathology. 108(7): 818-828. https://doi.org/10.1094/PHYTO-07-17-0255-R
19. Cardwell, K. F.; Cotty, P. J. 2002. Distribution of *Aspergillus* section Flavi among field soils from the four agroecological zones of the Republic of Bénin, West Africa. Plant Disease. 86(4): 434-439. https://doi.org/10.1094/PDIS.2002.86.4.434
20. Costa Baquião, A.; Martins Melo de Oliveira, M.; Alves Reis, T.; Zorzete, P.; Diniz Atayde, D.; Correa, B. 2013. Polyphasic approach to the identification of *Aspergillus* section Flavi isolated from Brazil nuts. Food Chemistry. 139(1-4): 1127-1132. https://doi.org/10.1016/j.foodchem.2013.01.007
21. Cotty, P. J. 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology. 79(7): 808-814.
22. Cotty, P. J. 1997. Aflatoxin-producing potential of communities of *Aspergillus* section Flavi from cotton producing areas in the United States. Mycological Research. 101(6): 698-704. https://doi.org/10.1017/S0953756296003139
23. Cotty, P. J.; Probst, C.; Jaime García, R. 2008. Etiology and management of aflatoxin contamination. In Mycotoxins: detection methods, management, public health and agricultural trade (Issue May: p. 287-299). CABI. Oxfordshire. UK. https://doi.org/10.1079/9781845930820.0287
24. Da Motta, S.; Valente Soares, L. M. 2000. Simultaneous determination of tenuazonic and cyclopiazonic acids in tomato products. Food Chemistry. 71(1): 111-116. https://doi.org/10.1016/S0308-8146(00)00040-6
25. De Fina, A. 1973. Mapa nacional de los distritos agroclimáticos argentinos. IDIA. 21-28.
26. De Oliveira Rocha, L.; Reis, G. M.; Braghini, R.; Kobashigawa, E.; de Araújo, J.; Corrêa, B. 2012. Characterization of aflatoxigenic and non-aflatoxigenic strains of *Aspergillus* section Flavi isolated from corn grains of different geographic origins in Brazil. European Journal of Plant Pathology. 132(3): 353-366. https://doi.org/10.1007/s10658-011-9881-4
27. De Rossi, R. L.; Guerra, F. A.; Brücher, E.; Torrico, A. K.; Maurino, M. F.; Lucini, E.; Gimenéz Pecci, M. P.; Plaza, M. C.; Guerra, G. D.; Camiletti, B. X.; Ferrer, M.; Laguna, I. G. 2017. Enfermedades del maíz de siembra tardía causada por hongos. In L. Borras; S. Uhart (Eds.), El mismo maíz, un nuevo desafío: Compendio primer congreso de maíz tardío. Dow Agrosciences. 109-127.
28. Devi Prameela, T.; Prabhakaran, N.; Kamil, D.; Borah, J.; Alemayehu, G. 2013. Development of SCAR marker for specific detection of *Aspergillus flavus*. African Journal of Microbiology Research. 7(9): 783-790. https://doi.org/10.5897/AJMR12.1552
29. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. W. 2021. InfoStatversión 2020. Centro de Transferencia InfoStat. FCA. Universidad Nacional de Córdoba. Argentina. www.infostat.com.ar
30. Donner, M.; Lichtemberg, P. S. F.; Doster, M.; Picot, A.; Cotty, P. J.; Puckett, R. D.; Michailides, T. J. 2015. Community structure of *Aspergillus flavus* and *A. parasiticus* in major almondproducing areas of California, United States. Plant Disease. 99(8): 1161-1169. https://doi.org/10.1094/PDIS-05-14-0450-RE
31. Dorner, J. W.; Horn, B. W.; Cole, R. J. 2000. Non-toxigenic strain of *Aspergillus oryzae* and *Aspergillus sojae* for biocontrol of toxigenic fungi. United States Department of Agriculture Patents N°6,027,724. https://patentimages.storage.googleapis.com/2b/7e/3e/f35408d90a6b8b/US6027724.pdf
32. Doster, M. A.; Cotty, P. J.; Michailides, T. J. 2014. Evaluation of the atoxigenic *Aspergillus flavus* strain AF36 in Pistachio orchards. Plant Disease. 98(7): 948-956. https://doi.org/10.1094/PDIS-10-13-1053-RE
33. Ezekiel, C. N.; Atehnkeng, J.; Odebode, A. C.; Bandyopadhyay, R. 2014. Distribution of aflatoxigenic *Aspergillus* section Flavi in commercial poultry feed in Nigeria. International Journal of Food Microbiology. 189: 18-25. https://doi.org/10.1016/j.ijfoodmicro.2014.07.026
34. FDA. Food and Drug Administration. 2011. Guidance for Industry: Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed. p. 1-15. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/guidance-industry-actionlevels-poisonous-or-deleterious-substances-human-food-and-animal-feed#afla
35. Folcher, L.; Delos, M.; Marengue, E.; Jarry, M.; Weissenberger, A.; Eychenne, N.; Regnault Roger, C. 2010. Lower mycotoxin levels in Bt maize grain. Agronomy for Sustainable Development. 30(4): 711-719. https://doi.org/10.1051/agro/2010005

36. Frisvad, J. C.; Hubka, V.; Ezekiel, C. N.; Hong, S. B.; Nováková, A.; Chen, A. J.; Arzanlou, M.; Larsen, T. O.; Sklenář, F.; Mahakarnchanakul, W.; Samson, R. A.; Houbraken, J. 2019. Taxonomy of *Aspergillus* section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Studies in Mycology. 93: 1-63. https://doi.org/10.1016/j.simyco.2018.06.001
37. Garcia Cela, E.; Gari Sanchez, F. J.; Sulyok, M.; Verheecke Vaessen, C.; Medina, A.; Krska, R.; Magan, N. 2020. Carbon dioxide production as an indicator of *Aspergillus flavus* colonization and aflatoxins/cyclopiazonic acid contamination in shelled peanuts stored under different interacting abiotic factors. Fungal Biology. 124(1): 1-7. https://doi.org/10.1016/j.funbio.2019.10.003
38. Giorni, P.; Magan, N.; Pietri, A.; Bertuzzi, T.; Battilani, P. 2007. Studies on *Aspergillus* section Flavi isolated from maize in northern Italy. International Journal of Food Microbiology. 113(3): 330-338. https://doi.org/10.1016/j.ijfoodmicro.2006.09.007
39. Gomez Talquenca, G.; Lanza Volpe, M.; Setien, N.; Gracia, O.; Grau, O. 2023. Serological relationships among strains of grapevine leafroll-associated virus 4 reflect the evolutive behavior of its coat protein gene. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(1): 104-114. DOI: https://doi.org/10.48162/rev.39.100
40. Gonçalves, S. S.; Cano, J. F.; Stchigel, A. M.; Melo, A. S.; Godoy Martinez, P. C.; Correa, B.; Guarro, J. 2012. Molecular phylogeny and phenotypic variability of clinical and environmental strains of *Aspergillus flavus*. Fungal Biology. 116(11): 1146-1155. https://doi.org/10.1016/j.funbio.2012.08.006
41. González Salgado, A.; González Jaén, T.; Vázquez, T.; Patiño, B. 2011. Microbial toxins. In O. Holst (Ed.), Microbial Toxins: Methods and Protocols, Methods in Molecular Biology. 739(19). Humana Press. https://doi.org/10.1007/978-1-61779-102-4
42. Grubisha, L. C.; Cotty, P. J. 2015. Genetic analysis of the *Aspergillus flavus* vegetative compatibility group to which a biological control agent that limits aflatoxin contamination in U.S. crops belongs. Applied and Environmental Microbiology. 81(17): 5889-5899. https://doi.org/10.1128/AEM.00738-15
43. Horn, B. W.; Greene, R. L.; Sobolev, V. S.; Dorner, J. W.; Powell, J. H.; Layton, R. C. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii*. Mycologia. 88(4): 574. https://doi.org/10.2307/3761151
44. Horn, B. W.; Dorner, J. W. 1999. Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. Applied and Environmental Microbiology. 65(4): 1444-1449.
45. Hoyos Ossa, D. E.; Hincapié, D. A.; Peñuela, G. A. 2015. Determination of aflatoxin M1 in ice cream samples using immunoaffinity columns and ultra-high performance liquid chromatography coupled to tandem mass spectrometry. Food Control. 56: 34-40. https://doi.org/10.1016/j.foodcont.2015.03.011
46. Jamali, M.; Ebrahimi, M.; Karimipour, M.; Shams Ghahfarokhi, M.; Dinparas Djadid, N.; Kalantari, S.; Pilehvar Soltanahmadi, Y.; Amani, A.; Razzaghi Abyaneh, M. 2012. An insight into the distribution, genetic diversity, and mycotoxin production of *Aspergillus* section Flavi in soils of pistachio orchards. Folia Microbiologica. 57(1): 27-36. https://doi.org/10.1007/s12223-011-0090-5
47. Juvvadi, P. R.; Chivukula, S. 2006. Putative calmodulin-binding domains in aflatoxin biosynthesis-regulatory proteins. Current Microbiology. 52(6): 493-496. https://doi.org/10.1007/s00284-005-0389-z
48. Kebede, H.; Abbas, H. K.; Fisher, D.; Bellaloui, N. 2012. Relationship between aflatoxin contamination and physiological responses of corn plants under drought and heat stress. Toxins. 4(11): 1385-1403. https://doi.org/10.3390/toxins4111385
49. Klich, M. A. 2002. Identification of common *Aspergillus* species. Centraalbureau voor Shimmelcultures. Utrecht. The Netherlands. p.116.
50. Klich, M. A. 2007. Environmental and developmental factors influencing aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus*. Mycoscience. 48(2): 71-80. https://doi.org/10.1007/S10267-006-0336-2
51. Klingelhöfer, D.; Zhu, Y.; Braun, M.; Bendels, M. H. K.; Brüggmann, D.; Groneberg, D. A. 2018. Aflatoxin-Publication analysis of a global health threat. Food Control. 89: 280-290. https://doi.org/10.1016/J.FOODCONT.2018.02.017
52. Kocsubé, S.; Perrone, G.; Magistà, D.; Houbraken, J.; Varga, J.; Szigeti, G.; Hubka, V.; Hong, S. B.; Frisvad, J. C.; Samson, R. A. 2016. *Aspergillus* is monophyletic: Evidence from multiple gene phylogenies and extrolites profiles. Studies in Mycology. 85: 199-213. https://doi.org/10.1016/j.simyco.2016.11.006
53. Kumar, S.; Stecher, G.; Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for Bigger Datasets. Molecular Biology and Evolution. 33(7): 1870-1874. https://doi.org/10.1093/molbev/msw054
54. Larkin, M. A.; Blackshields, G.; Brown, N. P.; Chenna, R.; Mcgettigan, P. A.; McWilliam, H.; Valentin, F.; Wallace, I. M.; Wilm, A.; Lopez, R.; Thompson, J. D.; Gibson, T. J.; Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. Bioinformatics. 23(21): 2947-2948. https://doi.org/10.1093/bioinformatics/btm404

55. Leslie, J. F.; Summerell, B. 2006. The *Fusarium* laboratory manual. Blackwell Publishing Professionall. 387 p.
56. Liu, D.; Coloe, S.; Baird, R.; Pedersen, J. 2000. Rapid mini-preparation of fungal DNA for PCR. Journal of Clinical Microbiology. 38(1): 471.
57. MAGyP - Agroindustria. Ministerio de Agricultura Ganadería y Pesca. 2020. Características de la región Parque Chaqueño. http://forestoindustria.magyp.gob.ar/archivos/informacionpor-region/parque-chaqueno.pdf
58. MAGyP. Ministerio de Agricultura Ganadería y Pesca. 2021. Estimaciones agrícolas. http://datosestimaciones.magyp.gob.ar/reportes.php?reporte=Estimaciones
59. Martins, L. M.; Sant'Ana, A. S.; Pelegrinelli Fungaro, M. H.; Silva, J. J.; Da Silva do Nascimento, M.; Frisvad, J. C.; Taniwaki, M. H. 2017. The biodiversity of *Aspergillus* section Flavi and aflatoxins in the Brazilian peanut production chain. Food Research International. 94: 101-107. https://doi.org/10.1016/j.foodres.2017.02.006
60. Moreno, J. 2004. Estudio comparativo de *Aspergillus flavus* y *Aspergillus parasiticus* en la producción de aflatoxinas bajo diferentes condiciones de humedad y temperatura. UNAM. Facultad de Estudios Superiores de Posgrado. Cuautitlán Izcali, Estado de México.
61. O'Donnell, K.; Nirenberg, H.; Aoki, T.; Cigelnik, E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. Mycoscience. 41(1): 61-78. https://doi.org/10.1007/BF02464387
62. Perrone, G.; Gallo, A.; Logrieco, A. F. 2014a. Biodiversity of *Aspergillus* section Flavi in Europe in relation to the management of aflatoxin risk. Frontiers in Microbiology. 5(377): 1-5. https://doi.org/10.3389/fmicb.2014.00377
63. Perrone, G.; Haidukowski, M.; Stea, G.; Epifani, F.; Bandyopadhyay, R.; Leslie, J. F.; Logrieco, A. 2014b. Population structure and aflatoxin production by *Aspergillus* Sect. Flavi from maize in Nigeria and Ghana. Food Microbiology. 41: 52-59. https://doi.org/10.1016/j.fm.2013.12.005
64. Pildain, M. B.; Cabral, D.; Vaamonde, G. 2005. Poblaciones de *Aspergillus flavus* en maní cultivados en diferentes zonas agroecológicas de la Argentina, caracterización morfológica y toxigénica. RIA. 34(3): 3-19.
65. Pitt, J.; Hocking, A. 2009. Fungi and Food Spoilage. Springer US. https://doi.org/10.1007/978-0-387-92207-2
66. Presello, D. A.; Botta, G.; Iglesias, J. 2004. Podredumbres de espiga de maíz y micotoxinas asociadas. Idia Xxi. 4(6): 152-157. http://www.biblioteca.org.ar/libros/210728.pdf
67. Presello, D. A.; Oviedo, M.; Fernandez, M.; Iglesias, J.; Copia, P. 2016. Resistencia a podredumbres. RTA. 10(32): 29-32.
68. Probst, C.; Bandyopadhyay, R.; Cotty, P. J. 2014. Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. International Journal of Food Microbiology. 174: 113-122. https://doi.org/10.1016/j.ijfoodmicro.2013.12.010
69. Reis, T. A.; Baquião, A. C.; Atayde, D. D.; Grabarz, F.; Corrêa, B. 2014. Characterization of *Aspergillus* section Flavi isolated from organic Brazil nuts using a polyphasic approach. Food Microbiology. 42: 34-39. https://doi.org/10.1016/j.fm.2014.02.012
70. Ren, Y.; Jin, J.; Zheng, M.; Yang, Q.; Xing, F. 2020. Ethanol inhibits Aflatoxin B1 biosynthesis in *Aspergillus flavus* by up-regulating oxidative stress-related genes. Frontiers in Microbiology. 10. https://doi.org/10.3389/fmicb.2019.02946
71. Riba, A.; Bouras, N.; Mokrane, S.; Mathieu, F.; Lebrihi, A.; Sabaou, N. 2010. *Aspergillus* section Flavi and aflatoxins in Algerian wheat and derived products. Food and Chemical Toxicology. 48(10): 2772-2777. https://doi.org/10.1016/j.fct.2010.07.005
72. Rodrigues, P.; Santos, C.; Venâncio, A.; Lima, N. 2011. Species identification of *Aspergillus* section Flavi isolates from Portuguese almonds using phenotypic, including MALDI-TOF ICMS, and molecular approaches. Journal of Applied Microbiology. 111(4): 877-892. https://doi.org/10.1111/j.1365-2672.2011.05116.x
73. Rosada, L.; Sant'anna, J.; Franco, C.; Esquissato, G.; Santos, P.; Yajima, J.; Ferreira, F.; Machinski, M.; Corrêa, B.; Castro-Prado, M. 2013. Identification of *Aspergillus flavus* isolates as potential biocontrol agents of aflatoxin contamination in crops. Journal of Food Protection. 76(6): 1051-1055. https://doi.org/10.4315/0362-028X.JFP-12-436
74. Saitou, N.; Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4(4): 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
75. Singh, P.; Cotty, P. J. 2019. Characterization of *Aspergilli* from dried red chilies (*Capsicum spp.*): Insights into the etiology of aflatoxin contamination. International Journal of Food Microbiology. 289: 145-153. https://doi.org/10.1016/j.ijfoodmicro.2018.08.025
76. Tamura, K.; Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution. 10(3): 512-526. https://doi.org/10.1093/oxfordjournals.molbev.a040023
77. Toso, R. E.; Toribio, M. S.; Diesser, M.; Borello, A. B.; Ardoino, S. M. 2018. Affections in animals and humans due to ingestion or exposure to aflatoxins. Preventive measures to avoid toxic effects. Ciencia Veterinaria. 20(1): 51-67. https://doi.org/10.19137/cienvet-20182013

78. Uka, V.; Moore, G. G.; Arroyo Manzanares, N.; Nebija, D.; De Saeger, S.; Di Mavungu, J. D. 2017. Unravelling the diversity of the cyclopiazonic acid family of mycotoxins in *Aspergillus flavus* by UHPLC triple-TOF HRMS. Toxins. 9(1): 1-21. https://doi.org/10.3390/toxins9010035
79. Ul Hassan, Z.; Al Thani, R.; Atia, F. A.; Alsafran, M.; Migheli, Q.; Jaoua, S. 2021. Application of yeasts and yeast derivatives for the biological control of toxigenic fungi and their toxic metabolites. Environmental Technology & Innovation. 22. 101447. https://doi.org/10.1016/j.eti.2021.101447
80. Vaamonde, G.; Patriarca, A.; Fernández Pinto, V.; Comerio, R.; Degrossi, C. 2003. Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section flavi from different substrates in Argentina. International Journal of Food Microbiology. 88(1): 79-84. https://doi.org/10.1016/S0168-1605(03)00101-6
81. Vaamonde, G. 2008. Micotoxinas emergentes y re-emergentes: Ácido ciclopiazónico. XI Congreso Argentino de Micología.
82. Wu, F.; Stacy, S. L.; Kensler, T. W. 2013. Global risk assessment of aflatoxins in maize and peanuts: are regulatory standards adequately protective? Toxicological Sciences. 135(1): 251-259. https://doi.org/10.1093/toxsci/kft132

Acknowledgments

The authors thank PhD. Ricardo Comerio (EEA INTA Anguil, La Pampa, Argentina) and PhD. Boris Camiletti (FCA Universidad Nacional de Córdoba, Córdoba, Argentina), for the fungal reference isolates used in this study and PhD Verónica Trucco (IPAVE - CIAP - INTA, Córdoba, Argentina) for computer assistance with phylogenetic analysis.

Funding sources

This work was supported by a grant from the PE I 074 (Integrated Pest Management) and PE I 147 (Food Safety) of the Instituto Nacional de Tecnología Agropecuaria (INTA).

Weed control in different germination fluxes with pre-emergent herbicides on sugarcane straw under dry periods

Control de malezas en diferentes flujos de germinación a través de herbicidas preemergentes en aplicaciones sobre paja de caña de azúcar y períodos de seco

Paulo Vinicius da Silva [1*], Paulo Henrique Vieira dos Santos [2], Patricia Andrea Monquero [3], Elias Silva de Medeiros [1], Bruna Ferrari Schedenffeldt [4], Roque De Carvalho Dias [5], Estela Maris Inácio [6], Daniela Maria Barros [1], Pedro Antonio Vougoudo Salmazo [1], Pedro Jacob Christoffoleti [7], Munir Mauad [1]

Originales: *Recepción:* 29/07/2021 - *Aceptación:* 12/03/2024

Abstract

Preemergent herbicides are a frequent weed control strategy. Considering different crop germinative fluxes, these products must present long-lasting weed control. This study evaluated preemergent herbicides in different germination fluxes of *Merremia aegyptia*, *Mucuna aterrima* and *Ricinus communis* when applied to different quantities of straw and different simulated dry periods. The experiment was conducted in a 4 × 2 × 2 factorial design with four replications. The treatments included four dry periods (0, 30, 60, and 90 days), two straw quantities (0 and 10 t ha^{-1}), and two germination fluxes. The herbicides amicarbazone (1225 g ha^{-1}), imazapic (147 g ha^{-1}), sulfentrazone (800 g ha^{-1}), and tebuthiuron (900 g ha^{-1}) were applied for preemergence weed control, and germination flush fluxes were evaluated at 7, 14, 21, 28, and 35 days after emergence (DAE) while verifying plant dry mass. Amicarbazone controlled less than 80% of the studied species at the 90-day dry period in the presence of straw. Imazapic did not present control residue for any of the species analyzed. Sulfentrazone showed the same control pattern at all germination fluxes, regardless of the amount of straw. Tebuthiuron successfully controlled all species in the first germination flush, exceeding 80% regardless of the amount of straw. Herbicides associated with straw quantities and dry periods have a significant impact on *M. aegyptiaca*, *M. aterrima* and *R. communis*.

Keywords

amicarbazone • flush • germination • imazapic • precipitation • residue • straw • sulfentrazone • tebuthiuron

1 Universidade Federal da Grande Dourados. Faculdade de Ciências Agrarias. Caixa Postal 79.804-970. Dourados. MS. Brasil. * paulovsilva@ufgd.edu.br
2 Ourofino Agrociência. Fazenda Experimental Ouro Fino. Caixa Postal 14115-000. Guatapará. SP. Brasil.
3 Universidade Federal de São Carlos. Centro de Ciências Agrarias. Departamento de Recursos Naturais e Proteção Ambienta – DRNPA. Caixa Postal 13600-970. Araras. SP. Brasil.
4 Universidade Estadual Paulista. Faculdade de Ciências Agrárias e Veterinárias - Câmpus de Jaboticabal. Caixa Postal 14884-900. Jaboticabal. SP. Brasil.
5 Universidade Federal do Triângulo Mineiro. Campus Universitário de Iturama. Caixa Postal 38280-000. Iturama. MG. Brasil.
6 Instituto Master de Ensino Presidente Antônio Carlos. Caixa Postal 38444-128. Araguari. MG. Brasil.
7 Universidade de São Paulo. Escola Superior de Agricultura Luiz de Queiroz, ESALQ ESALQ. Caixa Postal 13418-900. Piracicaba. SP. Brasil.

Resumen

La aplicación de herbicidas preemergentes es una estrategia de control de malezas, sin embargo, estos productos deben presentar residualidad para el control de diferentes flujos germinativos. Este estudio tuvo como objetivo evaluar la eficacia de herbicidas preemergentes en diferentes flujos de germinación de *Merremia aegyptia*, *Mucuna aterrima* y *Ricinus communis*, cuando se aplican sobre diferentes cantidades de paja y diferentes períodos secos simulados. El experimento se realizó en un diseño factorial 4 × 2 × 2 con cuatro repeticiones, el tratamiento incluye cuatro períodos secos (0, 30, 60 y 90), dos cantidades de paja (0 y 10 t ha^{-1}) y dos flujos de germinación. Los herbicidas amicarbazona (1225 g ha^{-1}); imazapic (147 g ha^{-1}), sulfentrazona (800 g ha^{-1}) y tebuthiuron (900 g ha^{-1}), se aplicaron para el control de malezas antes de la emergencia, y los flujos de flujo de germinación se evaluaron a los 7, 14, 21, 28 y 35 días después de la emergencia de especies (DAE), mientras se verifica la masa seca. La amicarbazona presentó una reducción del control para todas las especies en los periodos secos más prolongados y presencia de paja. La amicarbazona mostró menos del 80% de control para todas las especies a los 90 días del período seco en presencia de paja. Imazapic no presentó residuo control para ninguna de las especies analizadas. Para sulfentrazona, la cantidad de paja no afectó el control de las malezas en diferentes flujos de germinación, mostrando el mismo patrón de control independientemente de la cantidad de paja, porcentajes de control superiores al 80% independientemente de la cantidad de paja. Los herbicidas asociados a cantidades de paja y periodos secos tienen impacto sobre las especies de malezas *M.aegyptia*, *M.aterrima* y *R. communis*.

Palabras claves:
amicarbazone • flush • germinación • imazapic • precipitación • residuo • paja • sulfentrazone • tebuthiuron

Introduction

Raw sugarcane straw (without preburning) on the soil surface promotes a favorable environment for seed germination and weed development, such as *Merremia aegyptia* (L.) Urb., *Mucuna aterrima* Piper & Tracy, and *Ricinus communis* L. (12, 13). In these productive systems in Brazil, these three weed species are popularly known as "the three M's" (MMM-castor bean, *morning glory,* and mucuna). These species are adapted to sugarcane production systems with straw deposition on the soil surface. These systems hinder weed control with herbicides, causing serious damage to sugarcane production (2, 5, 18, 28, 33).

In addition to favoring the establishment of these species, the straw that remains on the soil surface represents a physical barrier to the action of preemergent herbicides (29), that once intercepted by the straw, becomes vulnerable to volatilization and/or photodegradation (7, 17) before reaching the soil (9). Another important aspect is the permanence period of a product on the straw. In Brazilian sugarcane plantations, products are applied during the winter season, characterized by low rainfall, especially in the southeast region (23). In addition, the longer the herbicide stays in the straw, the more susceptible it will be to degradation, consequently decreasing its transport and bioavailability for weed control (9, 27, 29, 30).

Some specific physical-chemical characteristics of the herbicides may facilitate an efficient straw-soil transport of these products. This, in addition to high solubility in water, absence of photodegradation (being preferentially degraded by microorganisms), and low K_{ow} (octanol/water partition coefficient, *i.e.* not having lipophilic character), (10) constitute key features for a successful product. Some herbicides have these physical-chemical characteristics. Among these herbicides, amicarbazone, presents high water solubility of 4.6 g L^{-1} at pH 4-9 and a low K_{ow} (1.23); sulfentrazone, has medium water solubility of 780 mg L^{-1} at pH 7 and medium K_{ow} (9.8 at pH 7); imazapic, has high water solubility of 2.200 mg L^{-1} at 25°C and a low K_{ow} of 0.16; and tebuthiuron, high solubility in water of 2.500 ml L^{-1} at 25°C and a high K_{ow} of 67.1 (26).

Several studies have reported effective control results in dry periods with the use of amicarbazone, imazapic, sulfentrazone and tebuthiuron herbicides (6, 11, 20, 22, 23, 30). Based on the above, this study evaluated the efficacy of preemergent herbicides in different germination flushes of *M. aegyptia*, *M. aterrima,* and *R. communis* when applied on different quantities of straw and with different simulated dry periods.

Materials and methods

The study was conducted under a greenhouse in the Department of Natural Resources of the Federal University of Sao Carlos at the agricultural science campus. The experiment was replicated twice, June/July 2016 and June/July 2017.

The herbicides were applied in preemergence in a completely randomized design following a 4 × 2 × 2 factorial scheme with four replications. The variables were four dry periods, two quantities of straw, and two germination fluxes. These factors were adopted for each of the three weed species (*M. aegyptia*, *M. aterrima*, and *R. communis* L.) and the four herbicide treatments (amicarbazone, imazapic, sulfentrazone, and tebuthiuron), individually. The experimental units were composed of 25 L polyethylene pots filled with soil from the arable layer of an Eutrophic Red Latosol (table 1).

Table 1. Soil chemical analysis (0 to 20 cm).

Tabla 1. Análisis químico del suelo (0 a 20 cm).

pH	M.O.	P	K	Ca	Mg	Al+H	SB	CTC	V	Argil	Silt	Sand
($CaCl_2$)	**(g dm^{-3})**	**(mg dm^{-3})**	**(mmolc dm^{-3})**						**(%)**	**(g kg^{-1})**		
5.2	15	12	1.9	15	4	20	20.9	40.9	51	175	55	770

Unit: Al, H+Al, K, Ca, Mg, SB and CTC (mmolc dm^{-3}); P (resina) (mg dm^{-3}); V, clay, silt, sand (%).

Unidad: Al, H + Al, K, Ca, Mg, SB y CTC (mmolc dm^{-3}); P (resina) (mg dm^{-3}); V, arcilla, limo, arena (%).

After filling the pots, 0 and 10 t ha^{-1} of sugar cane straw ('RB966928' variety) were allocated on the pot surface. Then, the herbicides amicarbazone (1225 g ha^{-1}), imazapic (147 g ha^{-1}), sulfentrazone (800 g ha^{-1}), or tebuthiuron (900 g ha^{-1}) were applied using a CO_2 pressurized, constant-pressure spray with fan-type tips (XR 110.02) at a pressure of 2.0 x 10^5 Pa with a syrup volume of 200 L ha^{-1}. During applications, the temperature was 17.1°C, the relative air humidity was 85%, and the wind velocity was 0.2 m s^{-1}.

After treatment application, the pots were submitted to four different periods without rain (0, 30, 60, and 90 days after herbicide treatment). After these periods, the pots received a rainfall simulation of 30 mm (flow rate of 1 L min^{-1}). Finally, the pots stood for 72 hours, enough time for the straw to dry and be carefully removed.

After removing the straw, the weed species *M. aegyptia*, *M. aterrima,* and *R. communis* were individually and carefully planted in the pots at 5 cm depth, aiming for minimum soil turnover and five plants per pot. Concerning *M. aterrima,* mechanical scarification broke dormancy.

The germination flux factor consisted of two different weed sowing times in the same experimental unit (pot). The first flux occurred immediately after rainfall simulation for each of the four dry periods (0, 30, 60, and 90 DAT with no water). At 35 days after emergence, for each dry period and first germination flow, the weeds were cut and removed for dry mass analysis. At this moment, a new germination flow began. For this purpose, in the same experimental units, the weed species *M. aegyptia*, *M. aterrima*, and *R. communis* were re-sown. Thus, for each dry period and experimental unit, two germination fluxes were simulated. The first one was related to weed sowing immediately after a 30 mm rain simulation (for each dry period: 0, 30, 60, and 90 days), and the second germination flux was sown after the first flush of germination.

Weed control percentage at each germination flux and within each dry period was evaluated at 7, 14, 21, 28, and 35 days after emergence (DAE) where 0 (zero) corresponded to no injury and 100 corresponded to plant death (1).

At 35 DAE for each germination flux and within each dry period, weeds were cut, packed in cardboard bags, taken to a greenhouse, and stored at 60°C for 72 hours. After those periods, the samples were weighed. For data analysis, dry mass values were expressed as reduction percentages in relation to the control without herbicide.

Statistics consisted of the reparametrized version of the logistic model with three parameters (3, 26) (Eq. 1):

$$Y = D/\{1+exp[B(log\,X - log\,E)]\} \quad (1)$$

where:
Y = Control and Biomass Reduction percentages
X = dry period
D = maximum estimate of the response variable
Parameter *E* = dry days estimates at 50% response
B = slope of the curve fitting at the inflexion point.

All statistical analyses were performed in R software (2022). The *ggplot2* (33) and *drc* (25) packages were used for graphical presentation and for fitting the Equation 1 model, respectively.

Results

According to ALAM (1974) and Vanhala *et al.* (2004), weed control percentages of 81-90% are classified as very good and 91-100% as excellent. For flow 1, control and biomass reduction of *M. aegypta* with amicarbazone were below 80% at 90 days of drought and on straw (figure 1, page 78). The other treatments controlled more than 90%, regardless of the amount of straw. For amicarbazone, in flow 2 with straw, control of *M. aegypta* was superior to 80% at 0 and 30 dry periods, but at 60 and 90, it was under 80% (ineffective). In the application without straw, control and reduction of *M. aegypta* biomass for the same germination flow was lower than 40% only at 90 dry periods.

In flow 1, control and biomass reduction of *M. aterrima* with amicarbazone was less than 80%, only at 90 days of dry periods, regardless of the amount of straw. At 60 days of dry period biomass reduction was lower than 80%, while for flow 2, control and biomass reduction were greater than 80% at 0 dry periods with and without straw (figure 1, page 78).

At flow 1, control and biomass reduction of *R. communis* with amicarbazone was below 80% at 90 DAT of drought, with or without straw (figure 1, page 78). Control of *R. communis* at flow 2 with amicarbazone was greater than 80% in the 0 dry periods without straw. In flow 2, biomass reduction of *R. communis* with amicarbazone was inadequate, with percentages below 80% in all dry periods and amounts of straw. Control of *M. aegyptia* with imazapic at flow 1 was over 80% in all dry periods with straw in the biomass reduction was inadequate with percentages below 80% at 60 and 90 days. Flow 2 without straw showed control over 80% at the 0 dry period, while not exceeding 60% with straw. For flow 2, biomass reduction was less than 60% in all dry periods and amounts of straw (figure 1, page 78).

In flow 1, control and biomass reduction of *M. aterrima* with imazapic was greater than 80% at 0 dry period, while control of *R. communis* was greater than 80% in the dry period with and without straw, and biomass reduction was over 80% at 0, 30 and 60 days regardless of straw. Flow 2 showed biomass reduction under 80% in all dry periods and amounts of straw (figure 1, page 78).

Control of M. aegyptia for flow 1 was greater than 80% in all dry periods, regardless of the amount of straw. The reduction of biomass in flow 1 was greater than 80% at 0 and 30 dry periods with straw and 0, 30 and 60 dry periods without straw (figure 1, page 78). In flow 2, control was over 80% at 0 dry periods without straw, and biomass reduction was greater than 80% at 0 and 30 dry periods with and without straw.

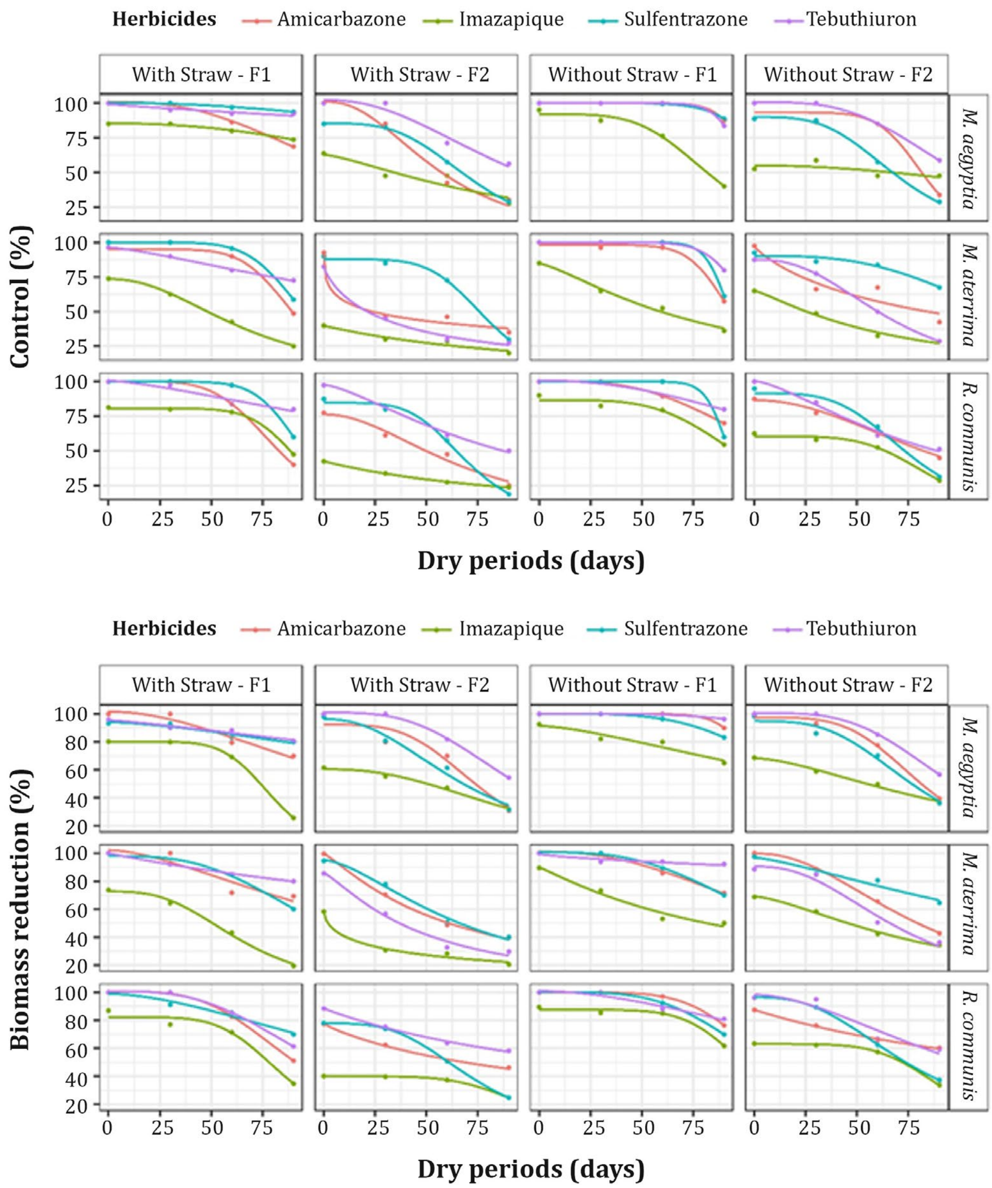


Figure 1. Control and biomass reduction of *Merremia aegyptia*, *Mucuna aterrima* and *Ricinus communis* at 35 DAE with amicarbazone; imazapic; sulfentrazone and tebuthiuron.

Figure 1. Control y reducción de biomasa de *Merremia aegyptia*, *Mucuna aterrima* and *Ricinus communis* a los 35 DAE a través de la amicarbazona; imazapic; sulfentrazona y tebutiuron.

Flow 1 with and without straw, and flow 2 without straw, showed control and biomass reduction of *M. aterrima* with sufentrazone over 80% at 0, 30 and 60 dry periods, regardless of the amount of straw. Flow 2 with straw, resulted in control and biomass reduction of *M. aterrima* over 80% at 0 and 30 dry periods, regardless of the amount of straw (figure 1). Flow 1, had control and biomass reduction of *R. communis* under 80% at the 90 dry period with and without straw. Control of *R. communis* in flow 2, achieved over 80% at 0 dry periods with straw, while without straw, control was superior to 80% at 0 and 30 dry periods. In flow 2, biomass reduction was greater than 80% at 0 and 30 dry periods, with and without straw (figure 1).

Control and biomass reduction of tebuthiuron at flow 1 was greater than 80% in all dry periods regardless of straw, while at flow 2, control and biomass reduction of thebuthiuron were greater than 80% at 0, 30 and 60 dry periods with or without straw (figure 1, page 78).

In flow 1, control and biomass reduction of *M. aterrima* was greater than 80% at 0 and 30 dry periods with straw, and 0, 30 and 60 dry periods without straw. For flow 1, biomass reduction of *M. aterrima* without straw exceeded 80% at all dry periods while at flow 2, this species control exceeded 80% at 0 and 30 dry periods, regardless of straw. Biomass reduction exceeded 80% at dry periods 0 and 30 without straw and dry period 0 with straw (figure 1, page 78). For flow 1, control of *R. communis* was over 80% in all dry periods with and without straw, while biomass reduction was less than 80% at 90 dry periods. In flow 2, control exceeded 80% at 0 and 30 dry periods, while biomass reduction was greater than 80% at the 0 dry period (figure 1, page 78).

Discussion

Weed control efficiency of amicarbazone was gradually reduced with longer dry periods and increasing amounts of straw. This reduction was higher at the 90 dry period and 10 t ha^{-1} sugarcane straw. Thus, it can be noted that longer dry periods and the presence of straw on the soil surface at the time of application, reduced the efficacy of amicarbazone. Contrasting results showed how 90 days after application resulted in over 90% control of *M. aterrima* (14).

Efficacy of pre-emergent amicarbazone over *I. grandifolia, B. plantaginea*, *B. decumbens,* and *C. rotundus* was reduced when applied on sugarcane straw, compared to bare soil applications (19). However, this herbide showed higher efficiencies when leached from the straw by simulated rain after application (19). These results are in agreement with our study, where longer dry periods associated with amicarbazone on straw resulted in reduced weed control efficiency, probably explained by amicarbazone having higher water solubility (4.6 g L^{-1}, pH 4-9) and low Kow (Log Kow of 1.23) (26). This contributes to low absorption and/or retention on straw and easier recovery of herbicide action by rain simulation. Thus, the higher control percentages during the first germination flux when the herbicide was directly applied to soil can be given by lower retention by straw and the consequent higher soil solution availability.

Studies on amicarbazone dynamics in sugarcane straw through HPLC/MS/MS showed that straw quantities equal to or greater than 5 t ha^{-1} retained almost all of the herbicide at the time of application, while increasing straw quantity (mainly at 15 and 20 t ha^{-1} sugarcane straw) reduced herbicide transport from straw to soil (9). The longer the period between herbicide application and the first rain, the lower the transport from straw to soil. However, 20 mm of rainfall at 7 and 14 days after application allowed enough recovery of the intercepted product.

Due to its high solubility, amicarbazone is easily washed from straw to soil. However, longer periods between product application on straw and the first rain may reduce product mobility, reducing weed control effectiveness. Amicarbazone´s solubility can also explain the lower control percentages obtained in the second germination flux, where greater leaching in the soil solution reduced herbicide quantity in the root zone. A second eventual factor related to the lower efficiency in the second germination flux is microbial degradation of amicarbazone influenced by soil humidity and higher temperatures.

The absent residual activity of imazapyr over a second weed emergence flux, regardless of species, dry periods, and/or straw quantities, constitutes a disadvantage considering the critical period of weed infestation in sugarcane exceeding 150 days after planting (21). Therefore, herbicides with prolonged residual activity within this period are more appropriate.

Long dry periods after application of preemergent herbicides resulted in the control efficient control of different species of morning glory (*Ipomoea purpurea*) (23). Control effectiveness of imazapic diminished by 40% between 30 and 60 days of dry periods after application on *M. aegyptia*, presumably due to the high solubility (2.200 mg L^{-1} at 25°C). In addition, this herbicide presents weak acid behavior, and low dissociation in the soil pH range between 5.0 and 7.0 (4, 15, 16). Since soil pH in this experiment was 5.2, dissociation and bioavailability of imazapic would be practically null (16). Additionally, the experimental units received simulations of daily and constant rainfall in the greenhouse solubilizing Imazapic. Other studies showed Imazapic applied to columns with clay soil and pH of 4.7 resulting in an average of approximately 46 and 23% phytotoxicity in cucumber plants at depths of 30 and 40 cm, respectively, through an 80 mm rainfall simulation, showing the high mobility of this herbicide in acidic soils (11). Therefore, interactions between dissociation and solubility of imazapic may have resulted in greater leaching and/or degradation of this herbicide, decreasing weed absorption in the sowing period. Finally, we must also consider the quantity of herbicide absorbed in the first germination flux. This reinforces the possible high mobility of the herbicide beyond the weed seeding zone, a possible reason for the absence of residual herbicide in a second weed germination flush.

Sugarcane straw did not influence sulfentrazone effects in the first and second weed germination fluxes (for all the plants), meaning control was similar in both quantities of straw (0 and 10 t ha^{-1}), regardless of the simulated dry period. This product efficiently controls levels in both the first and second germination fluxes. However, drought influenced weed control efficiency, since in general, control percentages decreased as dry periods increased. Difference abscence between applications may be related to the high solubility (490 mg L^{-1}) and low Kow (1.48) of sulfentrazone, inducing low interception and/or absorption of this herbicide in sugarcane straw, in addition to favoring a good recovery of sulfentrazone initially retained by straw. This behavior results in higher soil solution availability. These results are in agreement with those of Carbonari *et al.* (2016), who found that 20 mm of water released the maximum percentage of sulfentrazone, regardless of straw quantities. For the simulated dry period after sulfentrazone application, the authors obtained recoveries of 76.5, 61.7, and 42.3% for periods of 30 and 60 days after sulfentrazone application and rain simulation.

Tebuthiuron showed excellent control of the three evaluated weeds, *M. aegyptia*, *R. communis* and *M. aterrima*, in the first germination flux. However, a noticeable reduction in control efficiency was observed in the second weed germination flux when the product was positioned on sugarcane straw. The good tebuthiuron performance in the first and second weed germination fluxes may be related to its long half-life (up to 480 days), providing soil herbicide availability for proper control of a first germination flux and residual control of a second weed emergence flux. However, when tebuthiuron is applied on sugarcane straw during dry periods, higher amounts of rain are required for an adequate release from straw to soil.

Tebuthiuron applied at 5 or more t ha^{-1} straw resulted in almost 100% interception (29). The authors also found that lower quantities of straw resulted in higher output of the initially intercepted product. They also observed that for rainfall exceeding 20 mm, there is a tendency for the data to be similar, regardless of the quantity of straw. That is, maximum recovery capacity of the herbicide occurs with 20 mm of rain. Longer dry periods between tebuthiuron application on sugarcane straw and rainfall simulation result in less transport from straw to soil solution. The larger quantities of straw present on the soil surface at the moment of application resulted in greater interception of tebuthiuron. Additionally, longer dry periods between applications and rainfall simulation resulted in less herbicide recovery.

Conclusion

Amicarbazone herbicide presented effective control over the first weed germination flush. Straw quantity had an influence when associated with longer dry periods, while in the second germination flush, residual effects were affected by longer dry periods and the presence of straw. For Imazapic, the species presented variable control over the first germination flush, with residual effect. For Sulfentrazone, straw quantity did not have a significant influence on weed control. For Tebuthiuron, straw associated with longer dry periods reduced control percentages. However, in general, this herbicide presented enough weed control efficacy.

References

1. ALAM. 1974. Recomendaciones sobre unificación de los sistemas de evaluación en ensayos de control de malezas. Asociación Latinoamericana de Malezas. 35-38.
2. Azania, C. A. M.; Azania, A. A. P. M. 2009. Plantio da cana-de-açúcar e o manejo de plantas daninhas de difícil controle. PROTEC: Proteção de Plantas. 01: 30-33.
3. Barnes, E. R.; Lawrence, N. C.; Knezevic, S. Z.; Irmak, S.; Rodriguez, O.; Jhala, A. J. 2020. Dose response of yellow and white popcorn hybrids to glyphosate, a premix of 2, 4-D choline and glyphosate, or dicamba. Agronomy Journal. 112(4): 2956-2967. doi: https://doi.org/10.1002/agj2.20190
4. Burin, F.; Alves de Souza, C. M.; Ramires, I.; da Silva, P. V. 2022. Physical-chemical properties of spray syrup in tank-mixing multiple pesticides and water sources used in grain farming. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 132-144. DOI: https://doi.org/10.48162/rev.39.072
5. Campos, L. H. F.; Carvalho, S. J. P.; Nicolai, M.; Christoffoleti, P. J. 2016. Susceptibility of *Merremia cissoides*, *Neonotonia wightii* and *Stizolobium aterrimum* to the amicarbazone, imazapic and sulfentrazone herbicides. Revista Brasileira de Herbicidas. 15(2): 129-137. http://dx.doi.org/10.7824/rbh.v15i2.444
6. Carbonari, C. A.; Toledo, R. E. B.; Velini, E. D.; Negrisoli, E.; Correa, M. R.; Carbonari, C. V. S. R. 2009. Effects of different soil humidity conditions and germination depths of *Brachiaria plantaginea* and *Digitaria spp.* over amicarbazone herbicide efficacy. Revista Brasileira de Herbicidas. 8(3): 68-74. https://doi.org/10.7824/rbh.v8i3.70
7. Carbonari, C. A.; Velini, E. D.; Correa, M. R.; Negrisoli, E.; Rossi, C. V.; Oliveira, C. P. 2010. Efeitos de períodos de permanência de clomazone + hexazinona no solo e na palha de cana-deaçúcar antes da ocorrência de chuvas na eficácia de controle de plantas daninhas. Planta Daninha. 28(1): 197-205. https://dx.doi.org/10.1590/S0100-83582010000100023
8. Carbonari, C. A.; Gomes, G. L. G. C.; Trindade, M. L. B.; Silva, J. R. M.; Velini, E. D. 2016. Dynamics of Sulfentrazone Applied to Sugarcane Crop Residues. Weed Science. 64: 201-206. https://doi.org/10.1614/WS-D-14-00171.1
9. Cavenaghi, A. L.; Rossi, C. V. S.; Negrisoli, E.; Costa, E. A. D.; Velini, E. D.; Toledo, R. E. B. 2007. Dinâmica do herbicida amicarbazone (Dinamic) aplicado sobre palha de cana-de-açúcar (*Saccarum officinarum*). Planta Daninha. 25(4): 831-837. https://dx.doi.org/10.1590/S0100-83582007000400020
10. Christoffoleti, P. J.; López-Ovejero, R. F.; Damin, V.; Carvalho, S. J. P.; Nicolai, M. 2009. Comportamento dos herbicidas aplicados ao solo na cultura da cana-de-açúcar. Piracicaba. CP 2. 72p.
11. Correia, N. M.; Braz, B. A.; Fuzita, W. E. 2010. Eficácia de herbicidas aplicados nas épocas seca e úmida para o controle de *Merremia aegyptia* na cultura da cana-de-açúcar. Planta Daninha. 28(3): 631-642. https://doi.org/10.1590/S0100-83582010000300021
12. Da Silva, P. V.; Monquero, P. A.; Silva, F. B.; Bevilaqua, N. C.; Malardo, M. R. 2015a. Influence of sugar cane straw and sowing depth on the emergence of weed species. Planta Daninha. 33: 405-412. https://doi.org/10.1590/S0100-83582015000300003
13. Da Silva, P. V.; Monquero, P. A.; Munhoz, W. S. 2015b. Controle em pós-emergência de plantas daninhas por herbicidas utilizados na cultura da cana de açúcar. Revista Caatinga. 28(4): 21-32. https://doi.org/10.1590/1983-21252015v28n403rc
14. Da Silva, P. V.; Rodrigues Milagres Viana, H.; Andrea Monquero, P.; Moraes Ribeiro, N.; Pereira Neto, W.; Inacio, E. M.; Christoffoleti, P. J.; de Carvalho Dias, R. 2021. Influence of sugarcane (*Saccharum officinarum*) straw on weed germination control. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 53(1): 220-233. https://doi.org/10.48162/rev.39.021
15. Inoue, M. H.; Oliveira Jr., R. S.; Constantin, J.; Alonso, D. G. 2007. Potencial de lixiviação de imazapic e isoxaflutole em colunas de solo. Planta Daninha. 25(3): 547-555. https://doi.org/10.1590/S0100-83582007000300014
16. Kraemer, A. F.; Marchesan, E.; Avila, L. A.; Machado, S. L. O.; Grohs, M. 2009. Destino ambiental dos herbicidas do grupo das imidazolinonas: revisão. Planta Daninha. 27(3): 629-639. https://doi.org/10.1590/S0100-83582009000300025
17. Maciel, C. D. G.; Velini, E. D. 2005. Simulação do caminhamento da água da chuva e herbicidas em palhadas utilizadas em sistemas de plantio direto. Planta Daninha. 23(3): 471-481. https:// doi.org/10.1590/S0100-83582005000300011
18. Monquero, P. A.; Da Silva, P. V.; Hirata, A. C. S.; Martins, F. R. A. 2011. Monitoramento do banco de sementes de plantas daninhas em áreas com cana-de-açúcar colhida mecanicamente. Planta Daninha. 29(1): 107-119. https://doi.org/10.1590/S0100-83582011000100013
19. Negrisoli, E.; Rossi, C. V. S.; Velini, E. D.; Cavenaghi, A. L.; Costa, E. A.D.; Toledo, R. E. B. 2007. Weed control by amicarbazone applied in the presence of sugar-cane straw. Planta Daninha 25: 603-611. https://doi.org/10.1590/S0100-83582007000300021
20. Negrisoli, E.; Carbonari, C. A.; Corrêa, M. R.; Perim, L.; Velini, E. D.; Toledo R. E. B.; Victoria Filho, R.; Rossi, C. V. S. 2011. Efeitos de Diferentes condições de umidade do solo e profundidades de germinação de *Brachiaria plantaginea* e *Digitaria* spp. sobre a eficácia do herbicida tebuthiuron. Planta Daninha. 29: 1061-1068. https://dx.doi.org/10.1590/S0100-83582011000500013

21. Pitelli, R. A.; Durigan, J. C. 1985. Interferência de plantas daninhas em culturas agrícolas. Informe Agropecuário. 11(129): 16-27.
22. Prado, A. B. C. A.; Obara, F. E. B.; Brunharo, C. A. G.; Melo, M. S. C.; Christoffoleti, P. J.; Alves, M. C. 2013. Dinâmica de herbicidas aplicados em pré-emergência sobre palha de cana-de-açúcar em diferentes regimes hídricos. Revista Brasileira de Herbicidas. 12(2): 179-187. https://doi.org/10.7824/rbh.v12i2.211
23. Ribeiro, N. M.; Torres, B. A.; Ramos, S. K.; Dos Santos, P. H. V.; Simões, C. T.; Monquero, P. A. 2018. Differential susceptibility of morning glory (*Ipomoea and Merremia*) species to residual herbicides and the effect of drought periods on efficacy. Australian Journal of Crop Science.4: 1090-1098. http://10.21475/ajcs.18.12.07.PNE1013
24. R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. Austria. URL https://www.R-project.org/
25. Ritz, C.; Baty, F.; Streibig, J. C.; Gerhard D. 2015. Dose-Response Analysis Using R. PLoS ONE. 10(12): e0146021. doi: https://doi.org/10.1371/journal.pone.0146021
26. Rodrigues, B. N.; Almeida, F. S. 2018. 7ª ed. Guia de Herbicidas. Londrina. Edição dos autores. 764 p.
27. Rossi, C. V. S.; Velini, E. D.; Luchini, L. C.; Negrisoli, E.; Correa, M. R.; Pivetta, J. P.; Costa, A. G. F.; Silva, F. M. L. 2013. Dinâmica do herbicida metribuzin aplicado sobre palha de cana-de-açúcar (*Saccarum officinarum*). Planta Daninha. 31(1): 223-230. http://dx.doi.org/10.1590/S0100-83582013000100024
28. Silva, G. B. F.; Azania, C. A. M.; Novo, M. C. S. S.; Wutke, E. B.; Zera, F. S.; Azania, A. A. P. M. 2012. Tolerância de espécies de Mucuna a herbicidas utilizados na cultura da cana-deaçúcar. Planta Daninha. 30(3): 589-597. https://dx.doi.org/10.1590/S0100-83582012000300015
29. Tofoli, G. R.; Velini, E. D.; Negrisoli, E.; Cavenaghi, A. L.; Martins, D. 2009. Dinâmica do tebuthiuron em palha de cana-deaçúcar. Planta Daninha. 27(4): 815-821. https://dx.doi.org/10.1590/S0100-83582009000400020
30. Toledo, R. E. B.; Perim, L.; Negrisoli, E.; Corrêa, M. R.; Carbonari, C. A.; Rossi, C. V. S.; Velini, E. D. 2009. Eficácia do herbicida amicarbazone aplicado sobre a palha ou no solo no controle de plantas daninhas na cultura da cana-de-açúcar. Planta Daninha. 27(2): 319-326. https://dx.doi.org/10.1590/S0100-83582009000200015
31. Vanhala, P.; Kurstjens, D.; Ascard, J.; Bertram, A.; Cloutier, D. C.; Mead, A.; Rafaelli, M.; Rasmussen, J. 2004. Guidelines for physical weed control research: Flame weeding, weed harrowing and intrarow cultivation. In Proceedings of the 6th EWRS-Workshop on Physical and Cultural Weed Control. Lillehammer. Norway. 8-10 March. p. 208-239.
32. Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.
33. Zera, F. S.; Dias Júnior, A. B.; Azania, C. A. M.; Azania, A. A. P. M. 2012. Tolerância de *Luffa aegyptiaca* a herbicidas utilizados em cana-deaçúcar. STAB. 30: 50-52.

Acknowledgments

We would like to thank the São Paulo Research Foundation (FAPESP) for the financing and support of this research (Process: 2015/14833-0).

Bio-efficacy of entomopathogenic fungi and vegetable oils against the pink pineapple mealybug: *Dysmicoccus brevipes* (Cockerell)

Bioeficacia de hongos entomopatógenos y aceites vegetales contra el piojo harinoso rosado de la piña: *Dysmicoccus brevipes* (Cockerell)

Omara Pérez Panti [1], Rubén García de la Cruz [1*], Héctor González Hernández [2],
Saúl Sánchez Soto [1], Pedro Antonio Moscoso Ramírez [1], Francisco Izquierdo Reyes [1]

Originales: *Recepción:* 06/06/2023 - *Aceptación:* 11/03/2024

Abstract

Dysmicoccus brevipes (Cockerell) (Hemiptera: Pseudococcidae) is an important insect pest of pineapple worldwide due to the direct damage it causes and because it is a vector of mealybug pineapple wilt. Entomopathogenic fungi are an alternative management tool for this pest. A preliminary experiment evaluated the lethal effects of two isolates of *Beauveria bassiana* (BbCT, BbCa) and one isolate of *Metarhizium anisopliae* (Ma) against adult female *D. brevipes*. Subsequently, the efficacy of the most virulent isolates and a commercial strain of *Paecilomyces fumosoroceus* (PAE-SIN) were evaluated under laboratory and greenhouse conditions, either alone or in combination with soybean oil or neem oil. Results showed variation amongst isolates and that *B. bassiana* was the most effective. Isolate BbCa at 1×10^7 mL^{-1} conidia, was the most effective against *D. brevipes* nymphs and adults at 26 ± 1°C, causing 66% ± 6% mortality 8 days after inoculation. BbCa was the most virulent with an LC_{50} of 3.45×10^7 mL^{-1} conidia and a LC_{95} of 2.29×10^8 mL^{-1} conidia, under controlled conditions. Efficacy of BbCa increased when combined with neem oil, causing 100% mortality 6 days after inoculation. In conclusion, a combination of *B. bassiana* isolate BbCa and neem oil achieved 100% mortality in *D. brevipes* under the experimental conditions reported in this study.

Keywords
entomopathogenic fungi • biological control • pineapple • *Dysmicoccus* • vegetable oils

1 Colegio de Postgraduados Campus Tabasco. C. P. 86500. México.
[*] rubeng@colpos.mx
2 Colegio de Postgraduados Campus Montecillos. Institución de adscripción. Dirección Postal: 56230. México.

Resumen

Dismiccoccus brevipes (Cockerell) (Hemiptera: Pseudococcidae) es una de las plagas de la piña de mayor importancia a nivel mundial, no solo por los daños directos que ocasiona, sino por ser trasmisor del virus marchitez roja de la piña. Los hongos entomopatógenos son una alternativa para el manejo de este insecto. En un experimento preliminar se evaluó la patogenicidad y la virulencia de dos aislamientos de *Beauveria bassiana* (BbCT, BbCa) y uno de *Metarhizium anisopliae* (Ma), sobre hembras adultas de *D. brevipes.* Posteriormente, los aislamientos más virulentos y una cepa comercial de *Paecelomyces fumosoroceus* (PAE-SIN), fueron evaluados en otro experimento en condiciones de invernadero, solos o en combinación con aceite de soya y aceite de neem. Los aislamientos evaluados presentaron diferente grado de virulencia; sin embargo, *B. bassiana* resultó ser el más virulento. El aislamiento BbCa a una concentración inicial de 1×10^7 conidios mL^{-1} fue más efectivo contra adultos de *D. brevipes* comparado con el control, causando mortalidad del 66% ± 6% a los 8 días pos inoculación a 26 ± 1°C. BbCa presentó la mayor virulencia con una CL_{50}, de $3.45x10^7$ conidios mL^{-1} y una CL_{95} de $2.29x10^8$ conidios mL^{-1}, bajo condiciones controladas. Sin embargo, la eficacia se incrementó para BbCa, cuando se combinó con aceite de neem, al causar el 100 % de mortalidad a los 6 días pos inoculación. En conclusión, la combinación *B. bassiana* (BbCa) y aceite de neem fue el mejor tratamiento, con una mortalidad de 100% de *D. brevipes* bajo las condiciones experimentales reportadas en este estudio.

Palabras clave

entomopatógenos • control biológico • piña • *Dysmicoccus* • aceite vegetal

Introduction

Pineapple production generates significant economic resources worldwide. Mexico's main pineapple exports are destined for the United States market, with an annual value in 2020 of $30,602,000 USD (24). Unfortunately, the pineapple industry is affected by various phytosanitary problems. Since pineapple is grown intensively and in monoculture, pesticides are commonly applied for pest management, causing problems for human health, the environment, and agroecosystems. The mealybugs *Dysmicoccus brevipes* (Cockerell) (Hemiptera; Pseudococcidae) and *D. neobrevipes* Beardsley (Hemiptera: Pseudococcidae) are major pests of commercial pineapple cultivation (29) causing significant damage throughout the crop growth cycle; they are also vectors of Pineapple Mealybug Wilt associated Virus (PMWaV) which can cause up to 100% of export crop losses due to rejection of fruit (19). Recent management strategies for *D. brevipes* in pineapple are largely based on synthetic organophosphate insecticides. However, efficacy of chemical control is limited by the cryptic location and behavior of these insects on plants, and their waxy surface layer which is a barrier to the action of contact insecticides, even protecting eggs from residual effects. There is also increasing concern in general about the toxic risks of excessive pesticide use in agriculture. Therefore, exploration of economically viable and environmentally safe strategies is necessary. We hypothesize that commercial pineapple production could benefit from the use of botanical extracts and biological pest control agents, such as entomopathogenic fungi, within integrated pest management (33).

Entomopathogenic fungi can infect directly without the need for ingestion and so are effective against sucking pests such as aphids, mealybugs, whiteflies, and mosquitoes (4). Some entomopathogenic fungi have a combination of modes of action against arthropods: toxins; nutrient depletion; physiological disruption; and mechanical damage to internal tissues due to mycelium development (12). Efficacy of entomopathogenic fungi has been widely documented, particularly against mealybugs. For example, *Beauveria bassiana* (Bals.) Vuill. (Ascomycota: Hypocreales), *Lecanicillium lecanii* (Zimm.) and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota: Hypocreales) infect and kill *Paracoccus marginatus* Williams & Granara de Willinks (2). Despite this, there are few laboratory and field studies on management of *D. brevipes* using entomopathogenic fungi in pineapple. One study by Miranda Vindas and Blanco Getzler (2013) has evaluated a range of options in the laboratory that included both entomopathogenic fungi and botanical oil extracts that are known for their repellent, anti-feeding, and growth inhibition properties; high degradability; and environmental safety (18). Specific evaluations included: *B. bassiana* (4.0 x10^{10} conidia/g); *M. anisopliae* (1.0 x10^{10} conidia/g); a mixture of both fungi (0.5 g + 0.5 g/L distilled water, 4.0 x10^{10} conidia/g + 1.0 x10^{10} conidia/g); potassium salts; fatty acids (7 mL/L); and botanical extracts (a mixture of hot chili, garlic, onion, mustard and jackass bitters) (7 mL/L). Results showed high efficiency of entomopathogenic fungi and that the botanical extract achieved the fastest mortality. In the same publication, the botanical extract was also evaluated in a commercial pineapple plantation in comparison with the typical chemical control options Diazinon® 60 EC (diazinon) (0.5 ml/L) and Sevin® 80 WP (carbaryl) (1 kg/ha); the lowest incidence of mealybugs was achieved in the botanical extract treatment (16).

These results suggest that combinations of entomopathogenic fungi and vegetable oil extracts have potential as control agents that may increase mortality of adult *D. brevipes* females. For this reason, the potential of two native fungal isolates was evaluated in comparison with a commercial product based on *Paecelomyces fumosoroseus*. Our specific objectives were to determine the pathogenicity and virulence of the entomopathogenic fungi, alone and in combination with neem oil or soybean oil, against the pineapple mealybug under laboratory and greenhouse conditions.

Material and Methods

Experiments were done at the Biological Control Laboratory, Postgraduate College, Tabasco Campus, Cárdenas, Tabasco, Mexico, between January and December 2021.

Collection and mass rearing of *D. brevipes*

Dysmicoccus brevipes adults were collected from two varieties of pineapple (MD2 and bighead) on commercial plantations in Huimanguillo, Tabasco. Insects were taken to the Biological Control laboratory of the Postgraduate College, Tabasco Campus, for laboratory breeding, following the methods of Pandey & Johnson (2006). For the breeding stock, 50 eight-month-old pineapple cloves (25 bighead and 25 MD2 variety) were transplanted from a commercial pineapple plantation in Huimanguillo, Tabasco, into plastic pots and kept in a greenhouse at 30-35°C. Twenty days after potting, they were infested with adult female *D. brevipes* (20 per plant) in the greenhouse.

Entomopathogenic fungal isolates

Pathogenicity and virulence evaluations were made on two *Beauveria bassiana* (BbCa, BbCT) isolates and one isolate of *Metarhizium anisopliae* (Ma); all were native entomopathogenic isolates from Tabasco, Mexico held in the collection of the Biological Control Laboratory of the Colegio de Postgraduados, Tabasco Campus (table 1). Subsequent bio-efficacy experiments included a commercial product based on *Paecilomyces fumosoroceus* (PAE-SIN®).

Mycelia from each isolate was grown on sterile Sabouraud Dextrose Agar (ADS, Bioxon, Mexico) in Petri dishes, 90 x 15 mm for 3 weeks at 26±1°C in darkness. Conidia were then scraped from the surface and suspended in 0.03% Tween 80. Conidial concentration was determined using a Neubauer chamber, following the method of Inglis *et al.* (2012).

Table 1. Reference of the fungi used in the evaluation of pathogenicity against *D. brevipes.*

Tabla 1. Referencia de los hongos usados en la evaluación de patogenicidad contra *D. brevipes.*

Species	Key	Host	Location
Beauveria bassiana	BbCT	*Hypsiphilla grandella*	Huimanguillo, Tab. Mexico
Beauveria bassiana	BbCa	*Hypothenemus* sp.	Huimanguillo, Tab. Mexico
Metarhizium anisopliae	Ma	*Aeneolamia* sp.	Cárdenas, Tab. Mexico

Pathogenicity and virulence of *D. brevipes*

Pathogenicity and virulence of *B. bassiana* (BbCa, BbCT) and *M. anisopliae* (Ma) against *D. brevipes* were determined experimentally using a completely randomized design with four replicates of each treatment and control; the entire experiment was repeated on three occasions. Groups of ten adult females were each placed on two basal pieces of MD2 pineapple leaf (8 x 8 cm) inside a plastic box (20 cm x 10 cm x 10 cm) with openings covered with organdy mesh for ventilation. Wet filter paper was placed in each box to provide moisture. Each group of adults was sprayed (from a spray bottle) with 1.5 ml of conidia (either 10^6, 10^7 or 10^8 mL^{-1}) suspended in 0.05% aqueous Tween 80; the control was sprayed with 0.05% aqueous Tween 80 only. Applications were made following the methodology of Ramírez-Sánchez *et al.* (2019). Boxes containing treated insects were incubated at 26 ± 1°C, 65 -70% RH and a 14:10 h light: dark regime). Mortality was assessed daily for 8 days. Dead insects were incubated to determine cause of death (mycosis), following the methodology of Butt and Goettel (2000). Abbott´s formula was used to correct data for control mortality (1).

Bio-efficacy of B. bassiana and *P. fumosoroseus*, either alone or in combination with vegetable oils, against *D. brevipes* under greenhouse conditions

An experiment was set up under greenhouse conditions based on the results of the aforementioned bioassays. Pineapple suckers (variety MD2 [40 cm in size]) were planted individually in replicate pots, each containing 2 kg of sandy soil collected from a commercial pineapple plantation in Huimanguillo, Tabasco, Mexico. To each pot twenty *D. brevipes* adults were inoculated at the base of the pineapple sucker and incubated for one month before experimental treatments were added.

A total of eight treatments were compared including the highly virulent *B. bassiana* isolate, BbCa, and a formulated strain of *P. fumosoroseus* (PAE-SIN®), either alone or in combination with soybean oil (CARRIER)® or neem extract oil (Nimicide 80®) (table 2). All treatments were applied as 20 ml solutions/ suspensions; all fungal treatments contained 1 x 10^7 conidia mL^{-1}. There were four replicates of each treatment arranged in a completely randomized design. After inoculation, insect mortality was assessed daily for 8 days.

Table 2. Treatments evaluated in the greenhouse assay.

Tabla 2. Tratamientos evaluados en el experimento de invernadero.

Treatments	Key	Conidia mL^{-1}
B. bassiana	BbCa	1 x 10^7
P. fumosoroceus	PAE-SIN	1 x 10^7
Soybean oil	SO (CARRIER®)	1.2 mL^{-1} L water
Neem oil	NO (NIMICIDE 80®)	1 mL^{-1} L water
BbCa + SO	BbCa + SO	1 x 10^7
BbCa + NO	BbCa + NO	1 x 10^7
PAE-SIN + SO	PAE-SIN + SO	1.0 x 10^7
PAE-SIN + NO	PAE-SIN + NO	1.0 x 10^7
Control	Tween 80	0.05 %

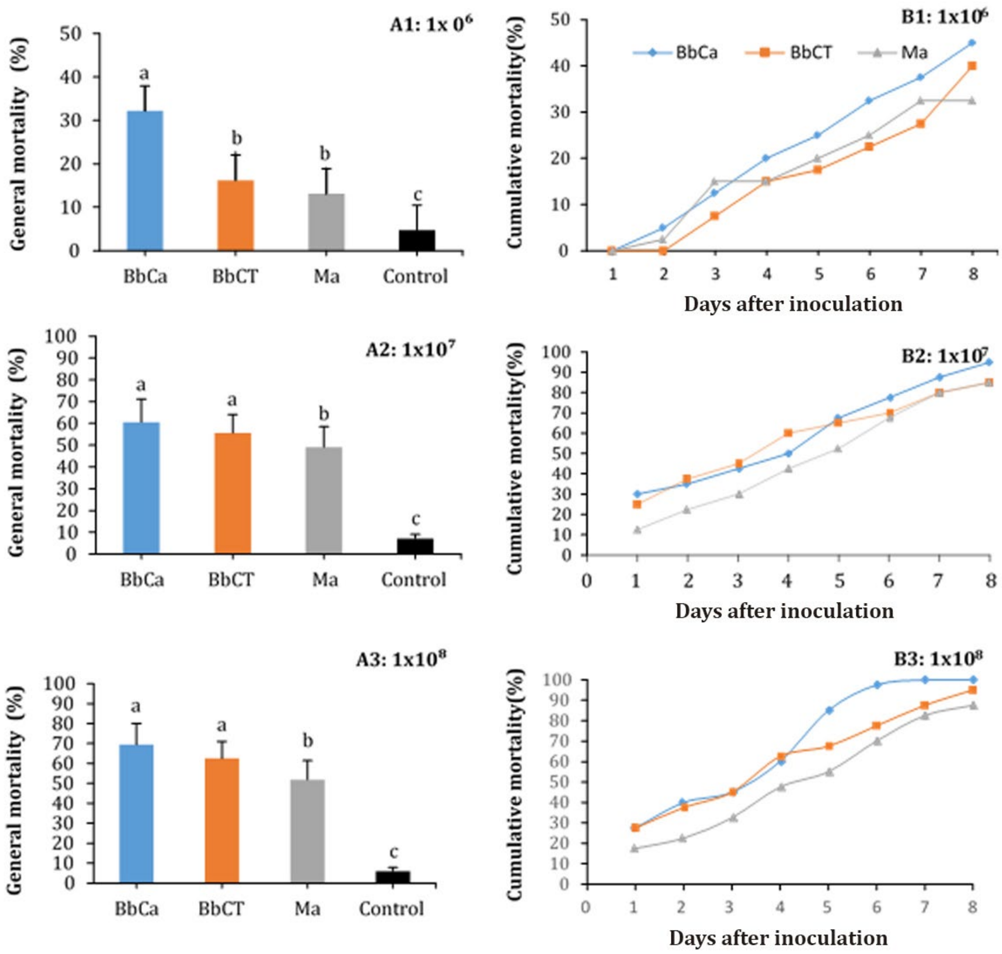
Statistical analysis

Probit analysis was used to estimate the LC_{50} and LC_{95} of each isolate with a 95% confidence limit. ANOVA and multiple comparisons of means for both isolates and their concentrations were also done with the Tukey test (p≤ 0.05) in SAS software (25). The probit regression model Φ^{-1} [Π(x)]=α+βx and the formula LC (P) = (qnorm (P) - α) / β were used to estimate lethal concentrations. Virulence graphs were constructed using R software v. 1.0.143 (22).

Results

Pathogenicity and virulence

Both isolates of *B. bassiana* (BbCa and BbCT) caused higher levels of *D. brevipes* mortality at all conidia concentrations evaluated compared with *M. anisopliae* at the same concentrations. There were highly significant differences amongst treatments ($p < 0.0001$) in the mean daily mortality after 8 days at the $1x10^6$ conidia concentration. The highest daily mean % mortality was achieved by isolate BbCa (32.2%), followed by isolate BbCT (16.3%) and then Ma (15.8%); in the control mortality was 4.7% (figure 1 A1). However, cumulative mortality at day 8 after inoculation was 45, 40, 32.5, and 4.5% for isolates BbCa, BbCT, Ma and the control treatment, respectively (figure 1 B1).



Bars with different letters are significantly different to each other (Tukey $p \leq 0.05$). Error bars represent ±1 standard error of the mean (SEM), n= 4.

Barras con diferentes letras son significativamente diferentes unas de otras (Tukey $p \leq 0,05$). Barras de error representan ±1 el estándar error de la media (SEM), n= 4.

Figure 1. Dynamics of A) general mean mortality for the period 1 - 8 days post inoculation and B) daily cumulative mortality of adult female *Dysmicoccus brevipes* after treatment with 1×10^6 (A), 1×10^7 (B) or 1×10^8 (C) conidia mL^{-1}, of *B. bassiana* (BbCa, BbCT) and *M. anisopliae* (Ma).

Figura 1. Dinámica de A) mortalidad media general del periodo de 1 - 8 días post inoculación y B) mortalidad acumulada diaria de hembras adultas de *Dysmicoccus brevipes* después de los tratamientos con 1×10^6 (A), 1×10^7 (B) o 1×10^8 (C) conidios mL^{-1}, de *B. bassiana* (BbCa, BbCT) y *M. anisopliae* (Ma).

Mortality data for the 1 x 10^7 conidia mL^{-1} concentration showed an increase in % mortality compared with the 1 x 10^6 conidia mL^{-1} concentration. There were highly significant differences amongst treatments ($p < 0.0001$); the highest mean daily % mortality over 8 days was achieved by isolates BbCa and BbCT, with 60% and 58.43% mortality, respectively, followed by isolate Ma with 49.06% and the control with 7.18% (figure 1 A2, page 87). Cumulative mortality on day 8 was 95, 85, 82, and 7.18% for isolates BbCa, BbCT, Ma and the control, respectively (figure 1 B2, page 87).

Mortality data for the 1x10^8 conidia mL^{-1} concentration showed an even greater increase in % mortality compared with the 1 x 10^6 and 1 x 10^7 conidia mL^{-1}. Highly significant differences among treatments ($p < 0.0001$) were detected, with the highest mean daily % mortality achieved by isolates BbCa and BbCT being 69.4 and 62.5%, respectively, followed by Ma (51.9%), while in the control mortality was 5.9% (figure 1 A3, page 87). Cumulative % mortality on day 8 was 100, 95, 87.5 and 7.1% for isolates BbCa, BbCT, Ma and the control, respectively (figure 1 B3, page 87).

Concerning cumulative mortality, *B. bassiana* isolate BbCa was more effective from day 5 post-inoculation than isolate BbCT. However, both isolates of *B. bassiana*, at each concentration evaluated, showed high efficacy against pineapple mealybug with increasing cumulative mortality over time after inoculation (figure 1B, page 87).

Proliferation of mycelium and conidial structures was observed on cadavers of *D. brevipes* produced during the first 4 days after inoculation. Abundant sporulation was detected from day 8 after inoculation, particularly from cadavers killed by *B. bassiana* isolates (figure 2).

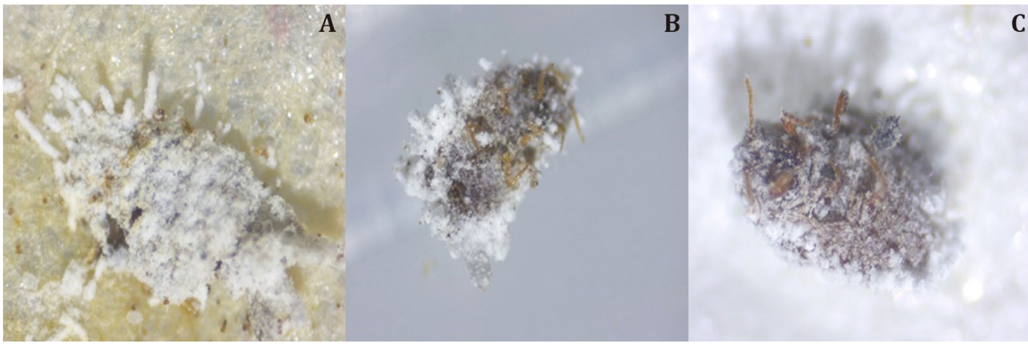


Figure 2. *Beauveria bassiana* isolates A) BbCa, B) BbCT and C) *Metarhizium anisopliae* (Ma) infecting adult female pineapple mealybug *Dysmicoccus brevipes* at 4 x magnification with an optical microscope.

Figura 2. Aislamientos de *Beauveria bassiana* A) BbCa, B) BbCT y C) *Metarhizium anisopliae* (Ma) infectando a hembras adultas del piojo harinoso de la piña *Dysmicoccus brevipes* a una magnificación de 4 x con un microscopio óptico.

Determination of virulence

LC_{50} values were 3.4 x 10^7, 5.2 x 10^7 and 8.5 x 10^7 mL^{-1} conidia for isolates BbCa, BbCT, and Ma, respectively. LC_{95} values were 2.29 x 10^8, 2.67 x 10^8 and 3.77 x 10^8 conidia mL^{-1} for isolates BbCa, BbCT and Ma, respectively (figure 3, page 89). Probit regression lines for *B. bassiana* were $Y = - 0.2864+8.401^{-09}$ (x) and $Y= - 0.3915+7.602^{-09}$(x), for BbCa, BbCT, respectively, whereas for *M. anisopliae*, it was $Y= - 0.4795+5.626^{-09}$(x), where 'Y' was the probit mortality and 'x' was the fungal concentration (figure 3, page 89). Data fitted well with the model and there was a positive correlation between conidial concentration evaluated and bioinsecticidal activity of both fungi.

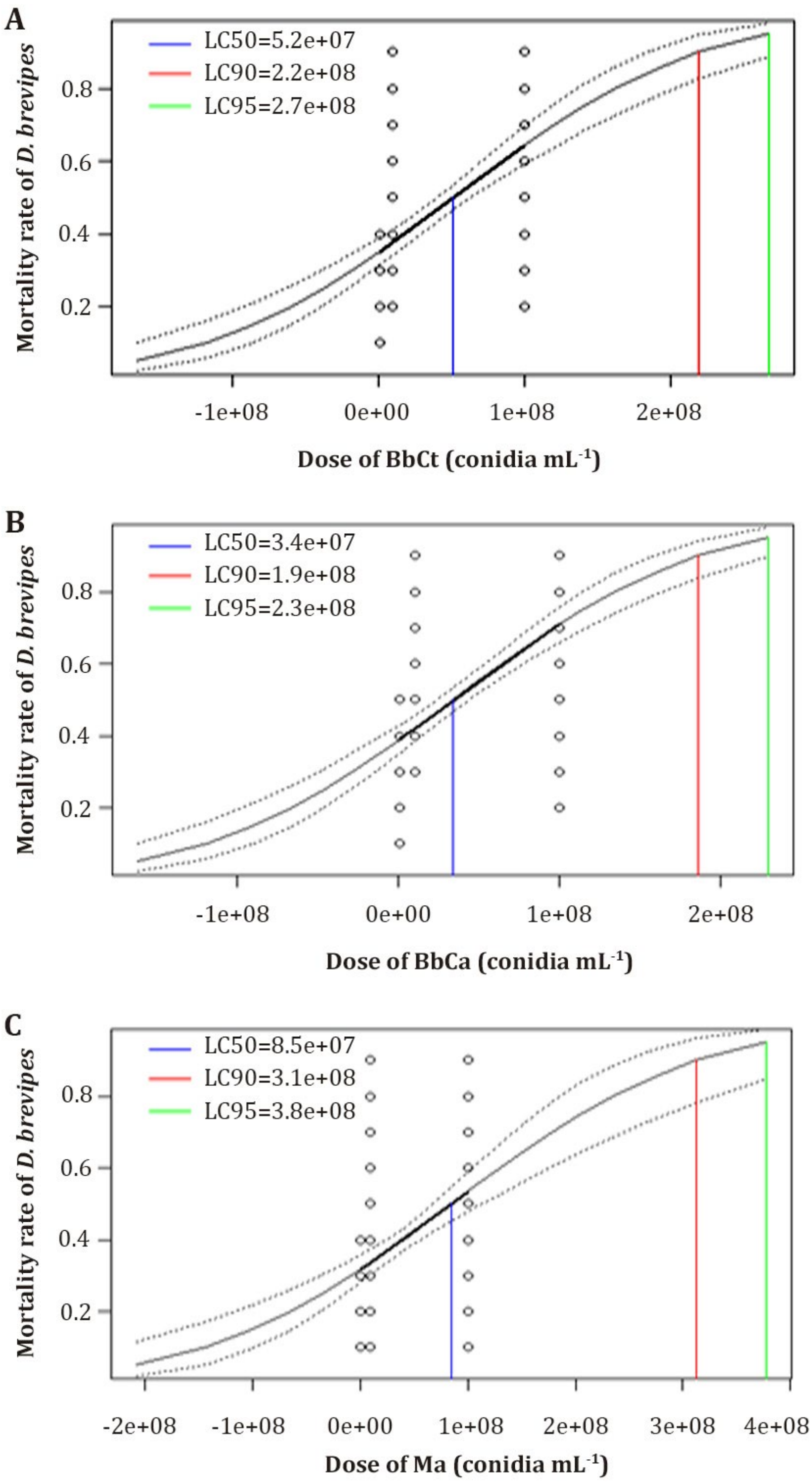


Figure 3. Corrected probit plot mortality of adult female *D. brevipes* treated with different conidia concentrations (log dose) of *B. bassiana* (BbCa [A], BbCT [B]) and *M. anisopliae* (Ma[C]) visualized using R software v. 1.0.143 (22).

Figura 3. Mortalidad probit corregida de hembras adultas de *D. brevipes* tratadas con diferentes concentraciones de conidios (log dosis) de *B. bassiana* (BbCa [A], BbCT [B]) y *M. anisopliae* (Ma[C]) visualizadas con el uso del programa R v. 1.0.143 (22).

Bio-efficacy of entomopathogenic fungi and vegetable oils against *D. brevipes*

Mortality of adult *D. brevipes* females following treatment with isolate BbCa (at 1 x 10^8 conidia mL^{-1}) or *P. fumosoroseus* alone or in combination with vegetable oils varied significantly amongst treatments ($p < 0.0001$). Mean mortality in the untreated control was 5.5%. The combination BbCa + neem oil achieved the highest mortality, 98.4%, ($p < 0.0001$) (figure 4). Neem oil treatment alone caused 81.4% mortality. The conidial concentrations of the commercial formulation of *P. fumosoroseus* used in this experiment, caused only 55.1% mortality. However, when *P. fumosoroseus* was combined with neem oil mortality was 66.1%.

Cumulative mortality data also showed that the combination of isolate BbCa and neem oil was sufficient to achieve high efficacy by day 6 after inoculation, which shortened the time to kill by 100% compared with neem oil alone.

Bars with different letters are significantly different to each other (Tukey p ≤ 0.05). Error bars represent ±1 standard error of the mean (SEM), n= 4.

Barras con diferentes letras son significativamente diferentes unas de otras (Tukey p ≤ 0,05). Barras de error representan ±1 el estándar error de la media (SEM), n= 4.

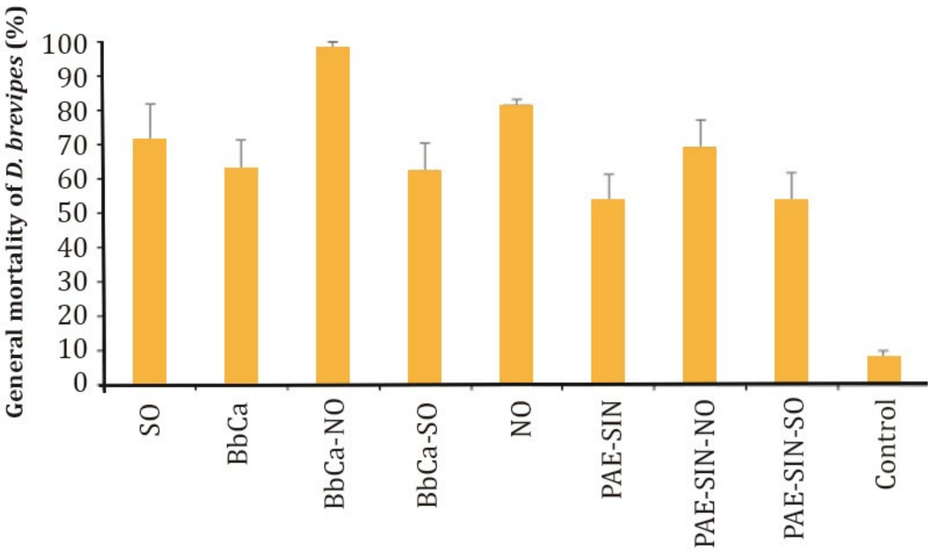


Figure 4. Overall mortality of *D. brevipes* after treatment with *B. bassiana* (isolate BbCa; 1 x 10^7 conidia mL^{-1}) or *P. fumosoroseus* at 1 x 10^7 conidia mL^{-1}(PAE-SIN®) either alone or in combination with soybean oil (SO) or neem oil (NO).

Figura 4. Mortalidad total de *D. brevipes* después de los tratamientos con *B. bassiana* (aislamiento BbCa; 1 x 10^7 conidios mL^{-1}) y *P. fumosoroseus* a 1 x 10^7 conidios mL^{-1} (PAE-SIN®) solos o en combinación con aceite de soya (SO) o aceite de neem (NO).

Discussion

In the present study isolates of both local native entomopathogenic fungi, *B. bassiana* and *M. anisopliae*, were pathogenic. They caused mortality and variable mycosis in adult female *D. brevipes*. The BbCa isolate was significantly more virulent than the *M. anisopliae* isolate under laboratory conditions. *B. bassiana* isolates are widely used as biological control agents of a wide variety of insect pests, including sucking pests such as mealybugs (21, 26, 32). For example, previous research showed that two isolates of *B. bassiana* (FF and PPRC-56) caused 97% and 100% mortality in adults of the mealybug *Paraputo ensete* (Williams and Ferrero) (Hemiptera: Pseudococcidae) twenty days after inoculation (14). In another study, two isolates of *B. bassiana* (GAR 17 B3, GB AR 23133) caused 67.5% and 64% mortality in the citrus mealybug *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) (5). Another isolate of *B. bassiana* caused 93% mortality in *Pseudococcus jackbeardsleyi* Gimpel & Miller (Hemiptera: Pseudococcidae) nymphs 5 days after treatment (10). The results of our research are in agreement with Mohamed (2016), who evaluated the virulence of *B. bassiana*, *M. anisopliae* and *Lecanicillium lecanii* isolates against adults of the mealybug, *Planococcus ficus* (Signoret) under laboratory conditions; they reported that virulence levels of *B. bassiana* were higher than those of *M. anisopliae* and *L. lecanii*, resulting in up to 98% mortality at a concentration of 5 x 10^8 mL^{-1} conidia. Moreover, Manjushree and Chellapan (2019) reported that isolates of *B. bassiana* caused higher mortality in *D. brevipes* (Cockerell) (Hemiptera: Pseudococcidae) at a concentration of 10^9 conidia mL^{-1} than isolates of *M. anisopliae* and *L. lecanii*. Like Surulivelu *et al.* (2012), Manjushree and Chellapan (2019) also reported that the same fungi were also effective against papaya mealybug *Paracoccus marginatus* (Williams) (Hemiptera: Pseudococcidae) under field conditions.

Concentration of inoculum (conidia) is a very important aspect of fungal pathogenicity and virulence. Results of our research suggest that isolate BbCa was the most virulent of the evaluated isolates with an LC_{50} of 3.4x10^7 conidia mL^{-1}, and that the higher the concentration of conidia the greater the mortality of *D. brevipes.*; overall 98% mortality was achieved 6 days after inoculation at 1 x10^7 conidia mL^{-1}. These results are consistent with other studies that report *B. bassiana* caused higher mortality of various mealybug species than *M. anisopliae* (11, 13). It has been reported that foliar applications of *V. lecanii* and *B. bassiana* (2×10^8 conidia mL^{-1}) in approximately 5 g/mL per L water is sufficient to reduce mealybug populations during months when the relative humidity is high (28). High effectiveness of *B. bassiana* and *P. fumosoroseus* against adult *D. brevipes* females was recorded from the 6th day after inoculation. In a field-level study, Ugalde-Trejos (2010) found no differences in the efficacy of *B. bassiana*, *M. anisopliae*, *Trichoderma* spp., and *Bacillus thuringiensis* treatments against populations of *D. brevipes* infesting pineapple.

Results of the bio-efficacy assay showed that mortality of *D. brevipes* increased when *B. bassiana* (isolate BbCa) and neem oil were combined, making it possible to consider this treatment for future field trials. Fernández and Juncosa (2002) reported that the use of adjuvant oils improved effectiveness of entomopathogenic fungi. In the same way, Elósegui and Elizondo (2010) found that mixtures of entomopathogenic fungi and adjuvants increased efficacy and tolerance of the product to wider ranges of temperatures. Vásquez, (2000) evaluated *in vitro* effectiveness of *B. bassiana*, *M. anisopliae*, *Entomophthora*, soap, hydrated lime, garlic extract (*Allium sativum*) and neem extract for control of *D. brevipes* in an organic pineapple plantation, where the greatest efficacy was achieved with mixtures of entomopathogenic fungi and extracts of soap, garlic, neem, and hydrated lime. Results of the present study agree with Miranda Vindas & Blanco Getzler (2013), who found that botanical extracts were highly efficient in causing *D. brevipes* mortality under field conditions. Gopal *et al.* (2021), found that the maximum cumulative mortality of *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) was achieved when entomopathogenic fungi *B. bassiana* + *L. lecanii* (6 g/L + 6 g/L) were applied together rather than individually, resulting in 57.6% mortality, while neem and pongamia vegetable oils at 15 mL/L caused cumulative mortality of 81.4%, compared with the standard dose of neem oil (10 g/L) which caused 78.1% mortality. Our results showed that neem oil in combination with entomopathogenic fungi such as *B. bassiana* was the most efficient in killing adult female *D. brevipes* in greenhouse tests with up to 100% mortality by day 8 post-inoculation.

Conclusion

Both local isolates of *B. bassiana* and *M. anisopliae* were pathogenic to adult female *D. brevipes*. When *B. bassiana* and *P*. *fumosoroseus* were combined with neem oil under greenhouse conditions bio-efficiency increased by 20 % and 11%, respectively. *B. bassiana* (BbCa) combined with neem oil resulted in the highest mortality of *D. brevipes* reaching up to 100% by 8 days after inoculation.

We suggest that more research is needed to evaluate effectiveness of entomopathogenic fungi in combination with vegetable oils under field conditions. Design of biocontrol programs against pineapple mealybug is recommended in pineapple-growing regions of Mexico, as a strategy within integrated pest management programs. This option could reduce the use of toxic insecticides, which are harmful to the environment and human health.

References

1. Abbott, W. S. 1925 A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 18: 265-267.
2. Amutha; Gulsar Banu 2017. Variation in mycosis of entomopathogenic fungi on mealybug, *Paracoccus marginatus* (Homoptera: Pseudococcidae). Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 87: 343-349.
3. Butt, T. M.; Goettel, M. S. 2000. Bioassays of Entomogenous Fungi. In: Navon, N., and & Ascher, K.R.S. (eds.). Bioassays of Entomopathogenic Microbes and Nematodes. CAB International. Wallingford. Oxon. UK. 141-195.
4. Cabanillas, H. E.; Jones, W. A. 2009. Pathogenicity of *Isaria* sp. (Hypocreales: Clavicipitaceae) against the sweet potato whitefly B biotype, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Crop Protection. 28(4): 333-337.
5. Chartier, V, C.; Hill, M. P.; Moore, S. D.; Dames, J. F. 2016. Screening of entomopathogenic fungi against citrus mealybug, *Planococcus citri* (Hemiptera: Pseudococcidae). African Entomology. 24(2): 343-351.
6. Elósegui. C. O.; Elizondo Silva, A. I. 2010. Evaluación microbiológica *in vitro* de mezclas de especies de hongos entomopatógenos ingredientes activos de bioplaguicidas cubanos. Fitosanidad. 14(2): 103-109.
7. Fernández, C.; Juncosa, R. 2002. Biopesticidas: la agricultura del futuro. *Phytoma*. 141: 14-19.
8. Ginting, S.; Djamilah, D.; Pamekas, T.; Bustaman, H.; Priyatiningsih, P.; Sipriyadi, S.; Wibowo, R. H. 2020. Pathogenicity of entomopathogenic fungi *Lecanicillium lecanii* and *Beauveria bassiana* against *Pseudococcus jackbeardsleyi* (Pseudococcidae) infecting rambutan. Serangga. 25: 1-11.
9. Gopal, G. S.; Venkateshalu, B.; Nadaf, A. M.; Guru, P. N.; Pattepur, S. 2021. Management of the grape mealy bug, *Maconellicoccus hirsutus* (Green), using entomopathogenic fungi and botanical oils: a laboratory study. Egyptian Journal of Biological Pest Control. 31(1): 1-8.
10. Inglis, G. D.; Enkerli, J.; Goettel, M. S. 2012. Laboratory techniques used for entomopathogenic fungi: Hypocreales. Manual of techniques in invertebrate pathology. 2: 18-53.
11. Lacey, L. A.; Frutos, R.; Kaya, H. K.; Vail, P. 2001. Insect pathogens as biological control agents: do they have a future? Biological Control. 21(3): 230-248.
12. Lacey, L. A.; Grzywacz, D.; Shapiro-Ilan, D. I.; Frutos, R.; Brownbridge, M.; Goettel, M. S. 2015. Insect pathogens as biological control agents: back to the future. Journal of Invertebrate Pathology. 132: 1-41.
13. Lemawork, S. 2008. Evaluation of entomopathogenic fungi and hot water treatment against root mealybug, *Cataenococcus ensete,* Williams and Matile-Ferrero (Homoptera: Pseudococcidae) Doctoral dissertation thesis, Department of Plant Sciences. Awassa College of Agriculture, School of Graduate Studies Hawassa University. Awassa. Ethiopia.
14. Lemawork, S.; Azerefegne, F.; Alemu, T.; Addis, T.; Blomme, G. 2011. Evaluation of entomopathogenic fungi against *Cataenococcus ensete* [Williams and Matile-Ferrero, (Homoptera: Pseudococcidae)]. *Crop Protection*. 30(4): 401-404.
15. Manjushree, G.; Chellappan, M. 2019. Evaluation of entomopathogenic fungus for the management of pink mealybug, *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae) on pineapple in Kerala. Journal of Entomology and Zoology Studies. 7: 1215-1222.
16. Miranda Vindas, A.; Blanco Metzler, H. 2013. Control de *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae), en el fruto de piña, San Carlos, Costa Rica. Agronomía Costarricense. 37(1): 103-111.
17. Mohamed, G. S. 2016. Virulence of entomopathogenic fungi against the vine mealy bug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae). Egyptian Journal of Biological Pest Control. 26(1): 47.
18. Oparaeke, A. M.; Dike, M. C.; Amatobi, C. I. 2005. Evaluation of botanical mixtures for insect pest management on cowpea plants. Journal of Agriculture and Rural Development in the Tropics and Subtropics. 106(1): 41-48.
19. Palma-Jiménez, M.; Blanco-Meneses, M.; Guillén-Sánchez, C. 2019. Las cochinillas harinosas (Hemiptera: Pseudococcidae) y su impacto en el cultivo de Musáceas. Agronomía Mesoamericana. 30(1): 281-298.
20. Pandey, R. R.; Johnson, M. W. 2006. Enhanced production of pink pineapple mealybug, *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae). Biocontrol Science and Technology. 16(4): 389-401.
21. Pelizza, S.; Mancini, M.; Russo, L.; Vianna, F.; Scorsetti, A. C. 2023. Control capacity of the LPSc 1067 strain of *Beauveria bassiana* (Ascomycota: Hypocreales) on different species of grasshoppers (Orthoptera: Acrididae: Melanoplinae), agricultural pests in Argentina. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(1): 98-103. DOI: https://doi.org/10.48162/rev.39.099
22. R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria: Available at: https://www. R-project. org/

23. Ramírez-Sánchez, C. J.; Morales-Flores, F. J.; Alatorre-Rosas, R.; Mena-Covarrubias, J.; Méndez-Gallegos, S. D. J. 2019. Efectividad de hongos entomopatógenos sobre la mortalidad de *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) en condiciones de laboratorio. Revista mexicana de ciencias agrícolas. 10(SPE22): 1-14.
24. SADER. 2021. Crece 16.2% producción de piña en México durante 2020. Secretaría de Agricultura y Desarrollo Rural(SADER). Lastaccess: August 8th, 2021. https://www.gob.mx/agricultura/prensa/ crece-16-2-produccion-de-pina-en-mexicodurante2020?idiom=es#:~:text=esquera%20 (SIAP). La%20Secretar%C3%ADa%20de%20Agricultura%20y%20Desarrollo%20Rural%20 report%C3%B3%20que%20M%C3%A9xico,comparaci%C3%B3n%20con%20el%20 a%C3%B1o%20previo
25. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Ver. 9. SAS Inst. Inc. Cary. N.C. USA.
26. Shah, P. A.; Pell, J. K. 2003. Entomopathogenic fungi as biological control agents. *Applied* Microbiology and Biotechnology. 61(5): 413-423.
27. Surulivelu, T.; Banu, J. G.; Rajan, T. S.; Dharajothi, B.; Amutha, M. 2012. Evaluation of fungal pathogens for the management of mealybugs in Bt cotton. Journal of Biological Control. 26(1): 92-96.
28. Tanwar, R. K.; Jeyakumar, P.; Monga, D. 2007. Mealybugs and their management Technical Bulletin 19. September 2007. National Centre for Integrated Pest Management LBS Building, Pusa Campus. New Delhi. 110(12): 1-10.
29. Torres, Á. A.; Aguilar Ávila, J.; Santoyo, C. V. H.; Uriza Á. D. E.; Zetina, L.; Rebolledo, M. A. 2018. La piña mexicana frente al reto de la innovación. Avances y retos en la gestión de la innovación. Colección Trópico Húmedo. Universidad Autónoma Chapingo. Chapingo, Estado de México. México. http://ciestaam.edu.mx/publicaciones2018/libros/pinia-mexicana-frente-alreto-de-la-innovacion.pdf.
30. Ugalde-Trejos, R. 2010. Evaluación a nivel de campo de la patogenicidad de microorganismos benéficos sobre poblaciones de cochinilla harinosa *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae), en el periodo posterior a la inducción floral del cultivo de piña (*Ananas comosus* (L.) MERR), en Finca Indaco Horquetas SA. Trabajo final de Graduación presentado a la Escuela de Agronomía como requisito parcial para optar el grado de Licenciatura en Ingeniería en Agronomía. Instituto Tecnológico de Costa Rica, Sede Regional San Carlos. Costa Rica.
31. Vásquez, A. O. L. 2000. Manejo de cochinilla (*Dysmicoccus brevipes*) en el cultivo de piña orgánica en la zona del Lago de Yojoa, Honduras. Proyecto especial presentado como requisito parcial para optar al título de Ingeniero Agrónomo en el Grado Académico de Licenciatura. Zamorano, Honduras.
32. Vianna, F.; Russo, L.; Troncozo, I.; Ferreri, N.; de Abajo, J. M.; Scorsetti, A. C.; Pelizza, S. 2023. Susceptibility of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) to the fungal entomopathogen Beauveria bassiana (Balsamo-Crivelli) Vuillemin s.l. (Hypocreales: Clavicipitaceae). Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 76-84. DOI: https://doi.org/10.48162/rev.39.110
33. Zart, M.; Ferracim de Macedo, M.; Santos, R. J. S.; Souza, D. G.; Pereira Brito, C.; de Souza Poletto, R.; Alves, V. S. 2021. Performance of entomopathogenic nematodes on the mealybug, *Dysmicoccus brevipes* (Hemiptera: Pseudococcidae) and the compatibility of control agents with nematodes. Journal of Nematology. 20(53): 1-10.

Acknowledgments

The authors would like to thank CONACYT (México) for the scholarship granted to the first author.

Application stages and doses of tembotrione herbicide in grain sorghum (*Sorghum bicolor*) crop

Etapas de aplicación y dosis del herbicida tembotrione en el cultivo del sorgo granífero (*Sorghum bicolor*)

Weverton Ferreira Santos [1], Alessandro Guerra da Silva [2*], Sérgio de Oliveira Procópio [3], Guilherme Braga Pereira Braz [2], Rafael Lopes Santos Rodrigues [2], Adriano Jakelaitis [1]

Originales: *Recepción:* 03/04/2023 **-** *Aceptación:* 15/03/2024

Abstract

The herbicide tembotrione is effective against grassy weeds constituting an important tool in sorghum crops. However, in Brazil, this herbicide is only registered for corn. This study aimed to evaluate the selectiveness of tembotrione combined with atrazine at different doses and developmental stages of grain sorghum. Two experiments were conducted in Rio Verde and Montividiu, state of Goiás, Brazil, in 2018. A randomized block design with four replications, in a 3x2+1+1 factorial arrangement, tested three developmental stages (V_3, V_5 and V_7), two doses of tembotrione (37.8 and 75.6 g ha^{-1}) combined with the herbicide atrazine (1,000 g ha^{-1}), an additional treatment only with atrazine at V_3 and a control, free of herbicide. Evident phytotoxicity was observed with the combination of tembotrione and atrazine at V_3 and V_5 stages. Symptoms included reductions in plant height, sorghum stem diameter, panicle length, and cumulative dry mass of sorghum plant shoots. However, there was no influence on thousand grains mass, regardless of application stages. Tembotrione at 37.8 g ha^{-1} combined with atrazine at 1,000 g ha^{-1} was selective for grain sorghum when applied at V_7, without affecting grain yield.

Keywords
atrazine • weeds • post-emergence • yield • *Sorghum bicolor*

1 Instituto Federal Goiano. Programa de Pós-graduação em Ciências Agrárias - Agronomia. Rodovia Sul Goiana. km 01. Zona Rural. Rio Verde. Goiás. Brasil. CEP: 75901-970.

2 Universidade de Rio Verde. Programa de Pós-graduação em Produção Vegetal. Fazenda Fontes do Saber. Campus Universitário. Caixa Postal 104, Rio Verde. Goiás. Brasil. CEP: 75901-970. * silvaag@yahoo.com.br

3 EMBRAPA Meio Ambiente. Jaguariúna. São Paulo. Brasil.

Resumen

El herbicida tembotrione tiene un buen control de malezas y puede ser una herramienta importante en el cultivo del sorgo. Sin embargo, este herbicida está registrado en Brasil solo para maíz. Este estudio tuvo como objetivo evaluar la selectividad de la tembotrione asociada a la atrazine en diferentes dosis y etapas de desarrollo del grano de sorgo. Se realizaron dos experimentos en Rio Verde-GO y Montividiu-GO, Brasil en 2018. Se adoptó un diseño de bloques al azar con cuatro repeticiones, en un factorial 3x2+1+1, con tres etapas de desarrollo (V_3, V_5 y V_7), dos dosis de tembotrione (37,8 y 75,6 g ha^{-1}) asociada al herbicida atrazine (1,000 g ha^{-1}), un tratamiento adicional con solo atrazine en V_3 más un testigo sin herbicida. Los resultados permitieron observar síntomas visibles de fitotoxicidad cuando se aplicó la asociación entre los herbicidas tembotrione y atrazine en la etapa V_3 y V_5. Los síntomas incluyeron reducciones en la altura de la planta y el diámetro del tallo del sorgo y reducciones en la longitud de la panícula con la aplicación en la etapa V_5. La aplicación de tembotrione en asociación con atrazine en V_3 y V_5 resultó en una reducción de la acumulación de masa seca en la parte aérea de las plantas de sorgo, pero sin influencia en la masa de mil granos, independientemente de la etapa de aplicación. El herbicida tembotrione en dosis de 37,8 g ha^{-1} en asociación con atrazine en dosis de 1,000 g ha^{-1} fue selectivo para sorgo en grano cuando se aplicó en V_7, sin causar reducción en el rendimiento de grano.

Palabras clave
atrazine • malezas • postemergencia • productividad • *Sorghum bicolor*

Introduction

Under water scarcity and considering food security, sorghum has become an increasingly strategic crop for both animal and human feed (9, 22, 24). In addition, sorghum has a significantly lower production cost than corn (10). Due to these factors, sorghum constitutes an option for second-crop cultivation, mainly in succession to soybean (15, 24).

Like sweet corn, sorghum greater sensitivity to herbicides constitutes one key factor limiting crop expansion (7, 20), especially considering herbicides effective against narrow-leaved weeds (grasses) (21). Evidence of this higher sensitivity refers to sorghum as an indicator plant for herbicide presence in soils (19) and substrates (6). This becomes more evident when analyzing the low number of herbicides registered for sorghum pre or postemergence in Brazil: 2,4-D, atrazine, S-metolachlor, imazethapyr, and imazapic. For imidazolinone herbicides, registers only include tolerant hybrids (17). In Brazil, no selective post-emergence product against weeds can ensure sorghum crops.

Tembotrione is registered in Brazil for post-emergence applications in corn (17). This herbicide is a triketone that inhibits the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), essential for carotenoid synthesis, causing leaf discoloration, necrosis, and plant death (12, 26). This highly selective herbicide for corn (11, 28) could be evaluated for sorghum, a taxonomically related species (Poaceae).

Studies on post-emergence herbicide selectivity should consider adequate dosages and application stages. According to Negrisoli *et al.* (2004), selectivity cannot solely be determined by isolated assessments of visual intoxication symptoms. These authors state that several herbicides reduce crop yield without causing visual injuries.

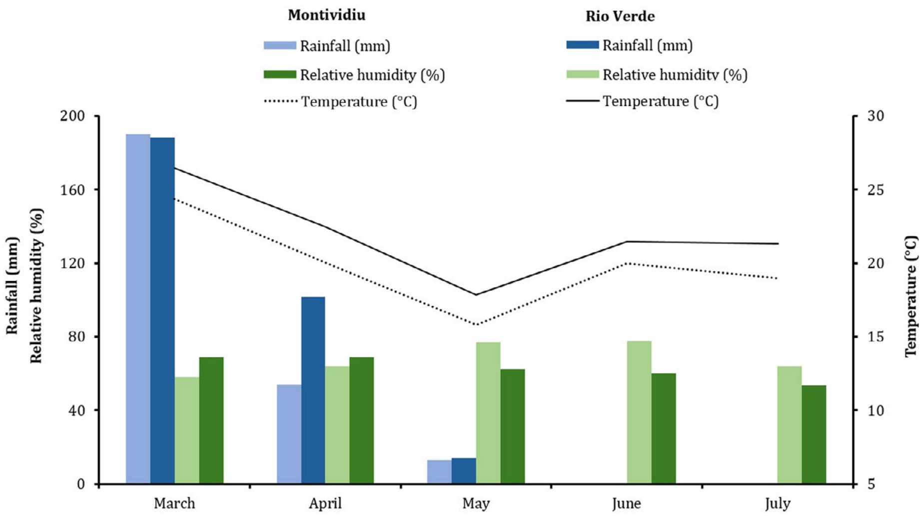
Complementing tembotrione with atrazine seeks sorghum selective improvement in weed control (8). In this sense, this research hypothesized that selectivity of the herbicide tembotrione in association with atrazine may vary depending on sorghum phenological stage and dose. The objective was to evaluate herbicide selectivity of tembotrione combined with atrazine, at post-emergence of sorghum in different doses and developmental stages.

Material and methods

Two field experiments were conducted in the southwest region of the state of Goiás, an important sorghum-producing region in Brasil, in the municipalities of Rio Verde (17°52'55" S; 50°55'43" W, 740 m altitude) and Montividiu (17°22'58" S; 51°22'40" W, 905 m altitude). The experiments were installed in the second crop after soybean in a no-till system, from March to July 2018.

Soils were classified as *Latossolo Vermelho-Amarelo Distrófico* and *Latossolo Vermelho distrófico* in Montividiu and Rio Verde, respectively (23). Soil analysis was performed for chemical and physical characterization at the 0.0 - 0.20 m layer. Results for Rio Verde were: pH in $CaCl_2$: 5.9; Ca, Mg, K, Al, H+Al: 4.2; 1.3; 0.2; 0.1; 4.3 in $cmol_c\ dm^{-3}$, respectively; P: 3.8 mg dm^{-3}; Organic matter (OM): 277 g dm^{-3}; clay, silt, and sand: 393; 125 and 482 in g kg^{-1}, respectively. In Montividiu results were: pH in $CaCl_2$: 5.6; Ca, Mg, K, Al, H+Al: 3.3; 0.9; 0.4, 0.05; 4.5 in $cmol_c\ dm^{-3}$, respectively; P: 37.6 mg dm^{-3}; OM: 226 g dm^{-3}; clay, silt, and sand: 249; 68 and 683 in g kg^{-1}, respectively.

According to the Köppen classification, the climate is tropical (Aw), with dry winter and rainfall concentrated in summer (2). Annual rainfall and temperature averages in Rio Verde and Montividiu are, 1,493 mm and 23.4°C and 1,512 mm and 23.0°C, respectively (3). Figure 1 shows meteorological data recorded during the experiments.



Source/Fuente: INMET - *Instituto Nacional de Meteorologia*. Collection station: Rio Verde (Goiás State).

Figure 1. Rainfall (mm), relative humidity (%) and average temperature (°C) in Rio Verde and Montividiu (Brazil), 2018 second crop.

Figura 1. Datos de precipitación (mm), humedad relativa del aire (%) y temperatura media (°C) durante los experimentos realizados en Rio Verde y Montividiu (Brasil), 2018 fuera de temporada.

The experiments were conducted in a randomized block design with four replications, in a 3x2+1+1 factorial arrangement, with three developmental stages (V_3, V_5 and V_7; with three, five and seven fully developed leaves, respectively), two doses of the herbicide tembotrione (37.8 and 75.6 g ha^{-1}) combined with the herbicide atrazine (1,000 g ha^{-1}), an additional treatment with only atrazine applied at V_3 stage and a herbicide-free control. Experimental units consisted of four 6.0 m long rows spaced 0.5 m, constituting a total area of 12.0 m^2 and a useful area of 5.0 m^2, with two central rows, ignoring the last 0.5 m.

In both locations, the grain sorghum hybrid BRS 330 (simple, early hybrid with red grains and no tannin) was used. This genotype is widely cultivated in the Southwest region of Goiás State. Mechanical sowing at 3 cm depth was carried out in March, with 200,000 plants per hectare. According to the regional cultivation system, no fertilization was carried out after the preceding soybean crop.

Herbicides were applied using a CO_2-pressurized backpack sprayer equipped with a bar with four double-fan nozzles, 110.02, with air induction, spaced 0.5 m apart. The working pressure was 2 kgf cm^{-2}, resulting in a spray volume of 150 L ha^{-1}. Mixture preparation included herbicide and an adjuvant based on soybean oil methyl ester at 0.1% v v^{-1}. Climate data during application (development stages V_3, V_5 and V_7, respectively) in Rio Verde were: temperature: 23.0; 23.8 and 28.3°C, relative humidity: 65; 56 and 53% and wind speed: 2.8; 2.4 and 2.5 m s^{-2} at V_3, V_5 and V_7, respectively. In Montividiu, the recorded data were temperature: 27.0; 28.8 and 29.6°C, relative humidity: 55; 59 and 39% and wind speed: 3.2; 1.2 and 2.1 m s^{-2}. Regardless of herbicide action, all plots were hand-weeded, leaving sorghum plants exposed to herbicide treatments. No pest or disease control was necessary.

Responses of sorghum plants to herbicide application, *i.e.* phytotoxicity, were visually evaluated at 2, 7, 14 and 28 days after application (DAA). According to the SBCPD scale (27), percentage scores were assigned where zero is symptoms absence and 100 is plant death. At harvest, five plants randomly chosen were evaluated for plant height (from neck to the upper end of the panicle); panicle length (between base and panicle tip); stem diameter (using a digital caliper, at the first node above the ground); and shoot dry mass (weighing oven-dried shoots, after forced air circulation at 65°C). In addition, evaluations were carried out for grain yield (harvesting and threshing panicles, with subsequent cleaning and weighing of grains) and thousand-grain mass, evaluated according to the Rules for Seed Analysis (1). For both variables, moisture was corrected to 13%.

Data were tested by ANOVA at 5% significance. Initially, phytotoxicity data were transformed by the expression ($\sqrt{x+1}$), for homoscedasticity and normality followed by Tukey test ($p \leq 0.05$) and Dunnet test ($p \leq 0.05$) for means comparison. Statistical tests were run using the Assistant statistical software (25).

Results and discussion

Treatments containing tembotrione and atrazine caused phytotoxicity symptoms in grain sorghum plants in both locations (table 1, page 98 and table 2, page 99). Observed symptoms included leaf discoloration (whitening), with a slightly reddish tint, and in most severe cases, necrosis, in accordance with Karam *et al.* (2009). At 2, 7 and 14 DAA and in both locations, the use of 75.6 g ha^{-1} tembotrione + atrazine, caused stronger effects compared to the lowest dose (37.8 g ha^{-1}) of tembotrione, also combined with atrazine. Furthermore, later applications (V_7 stage) resulted in lower phytotoxicity comparing V_3 and V_5 applications (table 1, page 98 and table 2, page 99).

At 28 DAA, the highest dose of tembotrione (75.6 g ha^{-1}) still showed the highest phytotoxicity (table 1, page 98 and table 2, page 99). However, a tendency towards recovery of sorghum plants was observed mainly in Rio Verde, compared to the previous evaluation at 14 DAA (table 1, page 98). In this evaluation, applications at V_3 caused greater phytotoxicity in sorghum than those at V_5 and V_7. Therefore, early applications of the combination of tembotrione and atrazine cause greater toxicity to sorghum plants, in accordance with Dan *et al.* (2010b), who reported that sorghum plants receiving tembotrione at three-leaf stage showed higher phytotoxicity than at five-leaf stage. This leads to the conclusion that, at more advanced stages, sorghum better tolerates tembotrione.

Atrazine alone (1,000 g ha^{-1} at V_3) did not cause any phytotoxicity in sorghum plants (table 1, page 98 and table 2, page 99), demonstrating its high selectivity when used at the said dose. Takano *et al.* (2018) found high selectivity even at higher doses (2,000 g ha^{-1}) when applied to grain sorghum plants with three to four leaves.

Increasing the dose of tembotrione from 37.8 to 75.6 g ha^{-1-} in combination with atrazine did not reduce plant height, regardless of the experimental location (table 3, page 100). Applications of tembotrione and atrazine herbicides at V_3 and V_5, in Rio Verde, induced lower growth rates, yielding smaller plants compared to the application at V_7. In Montividiu, V_3 application resulted in the highest occurrence of smaller plants. In agreement with Dan *et al.* (2010b), who concluded that tembotrione reduced growth when applied at V_3 stage.

Table 1. Visual phytotoxicity after application of tembotrione and atrazine herbicides at different doses and developmental stages of grain sorghum. Rio Verde (Brazil), 2018.

Tabla 1. Fitotoxicidad visual después de la aplicación de los herbicidas tembotriona y atrazina en diferentes dosis y estados de desarrollo de sorgo en grano. Rio Verde (Brasil), 2018.

Herbicides (g ha^{-1})	Developmental stages							
	V_3		V_5		V_7		Mean	
Phytotoxicity 2 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	5.0	*	7.5	*	4.2	*	5.5	a
Tembotrione + atrazine (75.6 + 1,000)	8.5	*	11.3	*	4.0	*	7.9	b
Means	6.7	B	9.3	C	4.1	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	15.0							
Phytotoxicity 7 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	14.7	*	10.5	*	5.2	*	10.1	a
Tembotrione + atrazine (75.6 + 1,000)	17.5	*	15.0	*	9.0	*	13.8	b
Means	16.1	C	12.7	B	7.1	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	5.2							
Phytotoxicity 14 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	11.2	*	16.2	*	6.5	*	11.3	a
Tembotrione + atrazine (75.6 + 1,000)	15.0	*	18.7	*	6.7	*	13.5	b
Means	13.1	B	17.5	C	6.6	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	10.7							
Phytotoxicity 28 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	4.0*	Aa	4.5*	Aa	4.0*	Aa	4.1	a
Tembotrione + atrazine (75.6 + 1,000)	10.0*	Bb	5.2*	Aa	4.2*	Aa	6.5	b
Means	7.0	B	4.8	A	4.1	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	8.4							

DAA: days after application. Means followed by different uppercases, in the same row, and lowercases, in the same column, are significantly different by Tukey's test (p≤0.05). * Significant difference from the control by Dunnett test (p≤0.05).

DAA: días después aplicación. Medias seguidas de diferentes letras mayúsculas, en la misma fila, y minúsculas, en la misma columna, son significativamente diferentes mediante prueba de Tukey (p≤0,05). * Diferencia significativa respecto al control mediante prueta de Dunnett (p≤0,05).

Table 2. Visual phytotoxicity after application of tembotrione and atrazine herbicides at different doses and developmental stages of grain sorghum. Montividiu (Brazil), 2018.

Tabla 2. Fitotoxicidad visual después de la aplicación de los herbicidas tembotriona y atrazina en diferentes dosis y estados de desarrollo de sorgo en grano. Montividiu (Brasil), 2018.

Herbicides (g ha^{-1})	Developmental stages							
	V_3		V_5		V_7		Means	
Phytotoxicity 2 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	8.7	*	6.2	*	3.2	*	6.0	a
Tembotrione + atrazine (75.6 + 1,000)	11.2	*	9.5	*	4.0	*	8.2	b
Means	10.0	B	7.8	B	3.6	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	14.6							
Phytotoxicity 7 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	12.2	*	9.5	*	4.5	*	8.7	a
Tembotrione + atrazine (75.6 + 1,000)	16.2	*	13.7	*	6.0	*	12.0	b
Means	14.2	B	11.6	B	5.2	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	11.2							
Phytotoxicity 14 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	22.5*	Ba	9.0*	Aa	7.7*	Aa	13.0	a
Tembotrione + atrazine (75.6 + 1,000)	31.2*	Cb	13.7*	Bb	7.7*	Aa	17.5	b
Means	26.8	C	11.3	B	7.7	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	8.2							
Phytotoxicity 28 DAA (%)								
Tembotrione + atrazine (37.8 + 1,000)	11.2*	Ba	3.7*	Aa	2.7*	Aa	5.9	a
Tembotrione + atrazine (75.6 + 1,000)	20.0*	Cb	6.2*	Bb	3.0*	Aa	9.7	b
Means	15.6	B	5.0	A	2.8	A		
Atrazine (1,000) at V_3	0.0							
Control	0.0							
Coefficient of variation (%)	9.3							

DAA: days after application. Means followed by different uppercases, in the same row, and lowercases, in the same column, are significantly different by Tukey's test (p≤0.05). * Significant difference from the control by Dunnett test (p≤0.05).

DAA: días después aplicación. Medias seguidas de diferentes letras mayúsculas, en la misma fila, y minúsculas, en la misma columna, son significativamente diferentes mediante prueba de Tukey (p≤0,05). * Diferencia significativa respecto al control mediante prueta de Dunnett (p≤0,05).

Table 3. Plant height and stem diameter after application of tembotrione and atrazine at different doses and developmental stages of grain sorghum. Rio Verde and Montividiu (Brazil), 2018.

Tabla 3. Altura de planta y diámetro de tallo después de la aplicación de tembotriona y atrazina en diferentes dosis y estados de desarrollo de sorgo en grano. Rio Verde y Montividiu (Brasil), 2018.

Herbicides (g ha^{-1})	Developmental stages							
	V_3		V_5		V_7		Means	
Plant height (cm)								
--- Rio Verde ---								
Tembotrione + atrazine (37.8 + 1,000)	108.2	*	111.1	*	119.1		112.8	a
Tembotrione + atrazine (75.6 + 1,000)	103.2	*	102.8	*	123.5		109.8	a
Means	105.7	B	106.9	B	121.3	A		
Atrazine (1,000) at V_3	124.3							
Control	126.9							
Coefficient of variation (%)	6.8							
--- Montividiu ---								
Tembotrione + atrazine (37.8 + 1,000)	113.4	*	120.5		119.9		117.9	a
Tembotrione + atrazine (75.6 + 1,000)	111.5	*	120.8		120.6		117.6	a
Means	112.4	B	120.6	A	120.2	A		
Atrazine (1,000) at V_3	123.2							
Control	122.3							
Coefficient of variation (%)	2.5							
Stem diameter (mm)								
--- Rio Verde ---								
Tembotrione + atrazine (37.8 + 1,000)	13.0		13.3		13.5		13.2	a
Tembotrione + atrazine (75.6 + 1,000)	11.9	*	13.3		13.3		12.8	a
Means	12.4	A	13.3	A	13.4	A		
Atrazine (1,000) at V_3	14.6							
Control	14.9							
Coefficient of variation (%)	9.5							
--- Montividiu ---								
Tembotrione + atrazine (37.8 + 1,000)	9.9	*	12.2		12.8		11.6	a
Tembotrione + atrazine (75.6 + 1,000)	9.7	*	13.0		12.6		11.8	a
Means	9.8	B	12.6	A	12.7	A		
Atrazine (1,000) at V_3	12.3							
Control	13.0							
Coefficient of variation (%)	11.3							

Means followed by different uppercases, in the same row, and lowercases, in the same column, are significantly different by Tukey's test ($p \leq 0.05$). * Significant difference from the control by Dunnett test ($p \leq 0.05$).

Medias seguidas de diferentes letras mayúsculas, en la misma fila, y minúsculas, en la misma columna, son significativamente diferentes mediante prueba de Tukey (p≤0,05). * Diferencia significativa respecto al control mediante prueta de Dunnett (p≤0,05).

In Rio Verde, only the treatment tembotrione + atrazine (75.6 + 1,000 g ha^{-1}) applied at V_3 reduced stem diameter of sorghum plants compared to the control without application and the treatment containing only atrazine (1,000 g ha^{-1}) (table 3). This was also observed in Montividiu, independently of the dose of tembotrione and atrazine applied at V_3. The reduction in stem diameter may cause lodging and consequent losses during mechanized harvesting.

In contrast, applications of tembotrione combined with atrazine at V_5 or V_7 did not change stem diameter (table 3). In popcorn, regardless of stage (V_2, V_4 or V_6), Maia *et al.* (2019) detected no differences in stem diameter after application of tembotrione + atrazine (76 + 2,000 g ha^{-1}).

Tembotrione affected panicle development in grain sorghum plants. In Rio Verde, tembotrione + atrazine (37.8 + 1,000 g ha^{-1}) applied at V_5, and tembotrione + atrazine (75.6 + 1,000 g ha^{-1}) at V_3 and V_5 reduced panicle length compared to atrazine alone (1,000 g ha^{-1}) and the control without herbicide (table 4). In Montividiu, this was observed only when sorghum plants were subjected to the combination of tembotrione + atrazine (75.6 + 1,000 g ha^{-1}) at the V_5, demonstrating that applications with these two herbicides at V_5 are not recommended.

Table 4. Panicle length and shoot dry mass after application of tembotrione and atrazine at different doses and developmental stages of grain sorghum. Rio Verde and Montividiu (Brazil), 2018.

Tabla 4. Longitud de panícula y masa seca de brotes después de la aplicación de tembotriona y atrazina en diferentes dosis y estados de desarrollo de sorgo en grano. Rio Verde y Montividiu (Brasil), 2018.

Herbicides (g ha^{-1})	Developmental stages							
	V_3		V_5		V_7		Means	
Panicle length (cm)								
--- Rio Verde ---								
Tembotrione + atrazine (37.8 + 1,000)	22.3		18.7	*	22.6		21.2	a
Tembotrione + atrazine (75.6 + 1,000)	20.9	*	17.7	*	23.0		20.5	a
Means	21.6	A	18.2	B	22.8	A		
Atrazine (1,000) at V_3	24.0							
Control	24.3							
Coefficient of variation (%)	5.8							
	--- Montividiu ---							
Tembotrione + atrazine (37.8 + 1,000)	21.0		20.9		21.4		21.1	a
Tembotrione + atrazine (75.6 + 1,000)	20.4		19.6	*	21.5		20.5	a
Means	20.7	A	20.2	A	21.4	A		
Atrazine (1,000) at V_3	22.1							
Control	22.5							
Coefficient of variation (%)	5.1							
Shoot dry mass (g $plant^{-1}$)								
--- Rio Verde ---								
Tembotrione + atrazine (37.8 + 1,000)	96.7	*	107.5	*	129.2		111.1	a
Tembotrione + atrazine (75.6 + 1,000)	91.5	*	103.0	*	124.5		106.3	b
Means	94.1	C	105.2	B	126.8	A		
Atrazine (1,000) at V_3	128.7							
Control	128.6							
Coefficient of variation (%)	2.30							
--- Montividiu ---								
Tembotrione + atrazine (37.8 + 1,000)	85.7	*	93.0	*	107.0		95.2	a
Tembotrione + atrazine (75.6 + 1,000)	82.7	*	91.2	*	106.7		93.5	a
Means	84.2	C	92.12	B	106.8	A		
Atrazine (1,000) at V_3	107.2							
Control	107.3							
Coefficient of variation (%)	6.20							

Means followed by different uppercases, in the same row, and lowercases, in the same column, are significantly different by Tukey's test ($p \leq 0.05$). * Significant difference from the control by Dunnett test ($p \leq 0.05$).

Medias seguidas de diferentes letras mayúsculas, en la misma fila, y minúsculas, en la misma columna, son significativamente diferentes mediante prueba de Tukey (p≤0,05). * Diferencia significativa respecto al control mediante prueta de Dunnett (p≤0,05).

Negative effects of tembotrione and atrazine herbicides at V_3 and V_5 include significant reductions in shoot dry mass in both locations (table 4, page 101). However, effects observed at V_3 were stronger than at V_5. On the other hand, applications of this mixture of herbicides at V_7 did not influence shoot dry mass. Thus, applications at more advanced stages result safer for sorghum plants. Dan *et al.* (2010a) found that tembotrione doses equal to or less than 118 g ha^{-1} applied to millet plants with seven leaves reduced shoot dry mass by less than 10%. Even focusing on grain yield, dry mass production is important for straw mulch, and the consequent no-till sustainability. Due to biomass production, the crop becomes an excellent option for crop rotation systems (16).

No dose of tembotrione (37.8 and 75.6 g ha^{-1}) + atrazine (1,000 g ha^{-1}), nor application stage (V_3, V_5 and V_7) affected the thousand-grain mass in either location (table 5, page 102-103). This yield component was hardly influenced by herbicides. Similarly, in corn, tembotrione at 100.8 g ha^{-1} at V_2, V_4, V_7 and V_{10}, did not affect thousand-grain mass (14).

In both locations, sorghum grain yield was significantly reduced with the application of tembotrione at V_3 or V_5, either at 37.8 or 75.6 g ha^{-1} combined with atrazine (table 5, page 102-103). On the other hand, applications of these herbicides at V_7 did not reduce grain yield, except for the treatment tembotrione at 75.6 + atrazine 1,000 g ha^{-1}, in Rio Verde. In this case, yield reduction was approximately 234 kg ha^{-1}.

Tembotrione selectivity is related to the cytochrome P-450 complex, majorly responsible for the metabolism of HPPD-inhibiting herbicides in species tolerant to active ingredients. In this study, sorghum grain yields indicated that tembotrione at 37.8 g ha^{-1}, in combination with atrazine at V_7, was selective when applied in the second crop in Southwestern Goiás.

The application of atrazine alone at V_3 did not reduce grain yield compared to the control without herbicide (table 5, page 102-103). Previous research states that post-emergence application of 2,000 g ha^{-1} atrazine is selective for sorghum, without affecting grain yield (21). Given reductions in grain yield after tembotrione doses over 75.6 g ha^{-1} combined with atrazine regardless of stage, using these herbicides becomes unfeasible. After lower reductions in yield when applying tembotrione to the most developed sorghum plants (five and seven developed leaves) (6), lower doses should be considered.

These evaluations should always be done in combination with atrazine, due to greater efficiency in weed control, even when used with higher doses (8). As grain sorghum is planted soon after soybean harvest, and grain losses are inevitable, atrazine becomes a fundamental herbicide for the control of volunteer plants of the legume when applied post-emergence (4).

Table 5. Thousand-grain mass and grain yield after application of tembotrione and atrazine at different doses and developmental stages of grain sorghum. Rio Verde and Montividiu (Brazil), 2018.

Tabla 5. Masa de mil granos y rendimiento de grano después de la aplicación de tembotriona y atrazina en diferentes dosis y estados de desarrollo de sorgo granífero. Rio Verde y Montividiu (Brasil), 2018.

Herbicides (g ha^{-1})	**Developmental stages**							
	V_3		**V_5**		**V_7**		**Means**	
Thousand grain mass (g)								
--- Rio Verde ---								
Tembotrione + atrazine (37.8 + 1,000)	23.6		23.3		24.7		23.9	a
Tembotrione + atrazine (75.6 + 1,000)	23.2		22.5		24.4		23.4	a
Means	23.4	A	22.9	A	24.5	A		
Atrazine (1,000) at V_3	24.2							
Control	24.4							
Coefficient of variation (%)	5.69							

Herbicides (g ha^{-1})	Developmental stages							
	V_3		V_5		V_7		Means	
--- Montividiu ---								
Tembotrione + atrazine (37.8 + 1,000)	25.1		25.0		25.1		25.1	a
Tembotrione + atrazine (75.6 + 1,000)	24.9		24.1		25.0		24.7	a
Means	24.0	A	24.6	A	25.1	A		
Atrazine (1,000) at V_3	25.5							
Control	25.7							
Coefficient of variation (%)	5.72							
Grain yield (kg ha^{-1})								
--- Rio Verde ---								
Tembotrione + atrazine (37.8 + 1,000)	2,765	*	2,644	*	3,456		2,955	a
Tembotrione + atrazine (75.6 + 1,000)	2,420	*	2,506	*	3,261	*	2,729	b
Means	2,592	B	2,575	B	3,358	A		
Atrazine (1,000) at V_3	3,478							
Control	3,495							
Coefficient of variation (%)	3.65							
--- Montividiu ---								
Tembotrione + atrazine (37.8 + 1,000)	2,232	*	2,573	*	3,192		2,666	a
Tembotrione + atrazine (75.6 + 1,000)	2,132	*	2,284	*	3,120		2,512	a
Means	2,182	C	2,428	B	3,156	A		
Atrazine (1,000) at V_3	3,350							
Control	3,381							
Coefficient of variation (%)	6.72							

Means followed by different uppercases, in the same row, and lowercases, in the same column, are significantly different by Tukey's test ($p \leq 0.05$). * Significant difference from the control by Dunnett test ($p \leq 0.05$).

Medias seguidas de diferentes letras mayúsculas, en la misma fila, y minúsculas, en la misma columna, son significativamente diferentes mediante prueba de Tukey ($p \leq 0{,}05$). * Diferencia significativa respecto al control mediante prueta de Dunnett ($p \leq 0{,}05$).

Conclusions

Phytotoxicity symptoms are more noticeable in grain sorghum plants when combinations of tembotrione and atrazine are applied at V_3 stage.

Reductions in plant height and stem diameter of grain sorghum plants are observed when tembotrione (37.8 and 75.6 g ha^{-1}) and atrazine (1,000 g ha^{-1}) are applied in combination at V_3 stage, while panicle reduction is evidenced when applications are at V_5.

Application of tembotrione (37.8 and 75.6 g ha^{-1}) and atrazine (1,000 g ha^{-1}) at V_3 and V_5 leads to a reduction in cumulative shoot dry mass of grain sorghum plants; with no effects on thousand-grain mass, regardless of application stage.

Tembotrione at 37.8 g ha^{-1} with atrazine at 1,000 g ha^{-1} is selective for grain sorghum (hybrid BRS 330) when applied at the V_7 stage, not reducing grain yield.

References

1. Brasil. Ministério da Agricultura, Pecuária e Abastecimento. 2009. Regras para análise de sementes. Brasília: Mapa/ACS. 399 p.
2. Cardoso, M. R. D.; Marcuzzo, F. F. N.; Barros, J. R. 2015. Classificação climática de Köppen-Geiger para o Estado de Goiás e o Distrito Federal. Acta Geográfica. 8: 40-55. DOI: https://doi.org/10.18227/2177-4307.acta.v8i16.1384
3. Climate-date. 2022. Dados climáticos para cidades mundiais. https://pt.climatedata.org/ (Access: 27/10/2022).
4. Dan, H. A.; Barroso, A. L. L.; Procópio, S. O.; Dan, L. G. M.; Oliveira Neto, A. M.; Guerra, N.; Braz, G. B. P. 2009. Controle químico de plantas voluntárias de soja Roundup Read®. Revista Brasileira de Herbicidas. 8: 96-101. DOI: https://doi.org/10.7824/rbh.v8i3.72
5. Dan, H. A.; Barroso, A. L. L.; Dan, L. G. M.; Oliveira Jr., R. S; Procópio, S. O.; Freitas, A. C. R.; Correa, F. M. 2010a. Seletividade do herbicida tembotrione à cultura do milheto. Planta Daninha. 28: 793-799. DOI: https://doi.org/10.1590/S0100-83582010000400012

6. Dan, H. A.; Barroso, A. L. L.; Dan, L. G. M.; Procópio, S. O.; Ferreira Filho, W. C; Menezes, C. C. E. 2010b. Tolerância do sorgo granífero ao herbicida tembotrione. Planta Daninha. 28: 615-620. DOI: https://doi.org/10.1590/S0100-83582010000300019
7. Dan, H. A; Dan, L. G. M.; Barroso, A. L. L.; Oliveira Jr., R. S.; Guerra, N.; Feldkircher, C. 2010c. Tolerância do sorgo granífero ao 2,4-D aplicado em pós-emergência. 28: 785-792. DOI: https://doi.org/10.1590/S0100-83582010000400011
8. Dourado Neto, D.; Martin, T. N.; Cunha, V. S.; Stecca, J. D. L.; Nunes, N. V. 2013. Controle de plantas daninhas no milho com o herbicida tembotrione. Enciclopédia Biosfera. 9: 808-817.
9. Elias, O. F. A. S.; Leite, M. L. M. V.; Azevedo, J. M.; Silva, J. P. S. S.; Nascimento, G. F.; Simplício, J. B. 2016. Características agronômicas de cultivares de sorgo em sistema de plantio direto no semiárido de Pernambuco. Ciência Agrícola. 14: 29-36. DOI: https://doi.org/10.28998/rca.v14i1.2318
10. Fialho, E. T.; Lima, J. A. F.; Oliveira, V.; Silva, H. O. 2002. Substituição do milho pelo sorgo sem tanino em rações de leitões: digestibilidade dos nutrientes e desempenho animal. Revista Brasileira de Milho e Sorgo. 1: 105-111.
11. Giraldeli, A. L.; Silva, G. S.; Silva, A. F. M.; Ghirardello, G. A.; Marco, L. R.; Victoria Filho, R. 2019. Efficacy and selectivity of alternative herbicides to glyphosate on maize. Revista Ceres. 66: 279-286. DOI: https://doi.org/10.1590/0034-737X201966040006
12. Karam, D.; Silva, J. A. A.; Pereira Filho, I. A.; Magalhaes, P. C. 2009. Características do herbicida tembotrione na cultura do milho. Sete Lagoas: Embrapa Milho e Sorgo. 6 p.
13. Maia, T. M.; Braz, G. B. P.; Machado, F. G.; Silva, A. G.; Andrade, C. L. L.; Simon, G. A. 2019. Associações herbicidas aplicadas na cultura do milho pipoca em diferentes estádios de desenvolvimento. Revista Brasileira de Milho e Sorgo. 18: 350-363.
14. Mançanares, L. B.; Gonçalves Netto, A.; Andrade, J. F.; Presoto, J. C.; Silva, L. J. F.; Carvalho, S. J. P. 2018. Seletividade de tembotrione aplicado em diferentes estádios fenológicos da cultura do milho safrinha. Revista Agrogeoambiental. 10: 65-73. DOI: 10.18406/2316-1817v10n420181167
15. Martins, L. S.; Menezes, C. B.; Simon, G. A.; Silva, A. G.; Tardin, F. D.; Gonçalves, F. H. 2016. Adaptabilidade e estabilidade de híbridos de sorgo granífero no sudoeste de Goiás. Agrarian. 9: 334-347.
16. Menezes, L. A. S.; Leandro, W. M.; Oliveira Júnior, J. P.; Ferreira, A. C. B.; Santana, J. G.; Barros, R. G. 2009. Produção de fitomassa de diferentes espécies, isoladas e consorciadas, com potencial de utilização para cobertura do solo. Bioscience Journal. 25: 7-12.
17. Ministério da Agricultura Pecuária e Abastecimento - Mapa. 2021. AGROFIT - Sistema de Agrotóxicos Fitossanitários. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Access: 25/03/2021.
18. Negrisoli, E.; Velini, E. D.; Tofoli, G. R.; Cavenaghi, A. L.; Martins, D.; Morelli, J. L.; Costa, A. G. F. 2004. Seletividade de herbicidas aplicados em pré-emergência na cultura de cana-de-açúcar tratada com nematicidas. Planta Daninha. 22:567-575. DOI: https://doi.org/10.1590/S0100-83582004000400011
19. Oliveira, T. L.; Senoski, M. P.; Pereira Assis, A. C. L. P.; Miranda, V. P.; Melo, C. A. D.; Reis, M. R. 2018. Seleção de espécies bioindicadoras do herbicida ethoxysulfuron. Revista de Ciências Agrárias. 61: 1-8.
20. Pataky, J. K.; Meyer, M. D.; Bollman, J. D.; Boerboom, C. M.; Williams, M. M. 2008. Genetic basis for varied levels of injury to sweet corn hybrids from three cytochrome P450- metabolized herbicides. Journal of the American Society for Horticultural Science. 133: 438-447. DOI: https://doi.org/10.21273/JASHS.133.3.438
21. Pimentel, G. V.; Guimarães, D. F.; Moreira, S. G.; Ávila, M. O. T.; Martins, I. A.; Bruzi, A. T. 2019. Selectivity and effectiveness of herbicides in the grain sorghum crop. Planta Daninha. 37: e019187771. DOI: https://doi.org/10.1590/S0100-83582019370100069
22. Queiroz, V. A. V.; Carneiro, H. L.; Deliza, R.; Rodrigues, J. A. S.; Vasconcellos, J. H.; Tardin, F. D.; Queiroz, L. R. 2012. Genótipos de sorgo para produção de barra de cereais. Pesquisa Agropecuária Brasileira. 47: 287-293. DOI: https://doi.org/10.1590/S0100-204X2012000200018
23. Santos, H. G.; Jacomine, P. K. T.; Anjos, L. H. C.; Oliveira, V. A.; Lumbreras, J. F.; Coelho, M. R.; Almeida, J. A.; Araujo Filho, J. C.; Oliveira, J. B.; Cunha, T. J. F. 2018. Sistema Brasileiro de Classificação de Solos. Brasília: Embrapa 5ª Ed. 356p.
24. Silva, A. G.; Francischini, R.; Goulart, M. M. P. 2015. Desempenho agronômico e econômico de híbridos de sorgo granífero na safrinha em Montividiu-GO. Revista de Agricultura. 90: 17-30.
25. Silva, F. A. S.; Azevedo, C. A. V. 2009. Principal Components Analysis in the Software Assistat-Statistical Attendance. In: World congress on computers in agriculture. 7. Reno-NV-USA: American Society of Agricultural and Biological Engineers.
26. Silva, R. A. A.; Oliveira, C. R.; Melo, C. A. D.; Mendes, K. F.; Reis, M. R. 2018. Residual effect of tembotrione in soil with distinct textures and humidity. Revista Brasileira de Ciências Agrárias. 13:e5594. DOI: 10.5039/AGRARIA.V13I4A5594
27. Sociedade Brasileira Da Ciência Das Plantas Daninhas. 1995. Procedimentos para instalação, avaliação e análise de experimentos com herbicidas. SBCPD: Londrina. 42 p.
28. Stephenson, D. O.; Bond, J. A.; Landry, R. L.; Edwards, H. M. 2015. Weed management in corn with postemergence applications of tembotrione or thiencarbazone: tembotrione. Weed Technology. 29: 350-358. DOI: https://doi.org/10.1614/WT-D-14-00104.1

29. Takano, H. K.; Kalsing, A.; Fadin, D. A.; Rubin, R. S.; Neves, R.; Marques, L. H. 2018. Chemical weed management in grain sorghum and selectivity of atrazine + S-metolachlor to different hybrids. Revista Brasileira de Milho e Sorgo. 17: 460-473. DOI: https://doi.org/10.18512/1980-6477/rbms.v17n3p460-473

Acknowledgments

Thanks to Federal Institute of Science and Technology (IFGoiano - Campus Rio Verde) and University of Rio Verde (UniRV) for funding and support to this research.

Traditional cow-calf systems of the northern region of Santa Fe, Argentina: current situation and improvement opportunities

Sistemas de cría tradicionales de la región norte de Santa Fe, Argentina: situación actual y oportunidades de mejora

Guillermina Gregoretti [1*], Javier Baudracco [2], Carlos Dimundo [1], Belén Lazzarini [1], Julieta Scarel [3], Agustín Alesso [2], Claudio Machado [4]

Originales: *Recepción:* 12/04/2023 - *Aceptación:* 29/02/2024

Abstract

Cow-calf systems are at the core of Argentina´s significant national beef industry. The objectives were: i) to characterize the productive state of traditional cow-calf systems, named BASE, from the northern region of Santa Fe province, ii) to identify technologies for the productive improvement of the BASE system, and iii) to quantify the productive and economic impact of the adoption of the identified technologies. To characterize the BASE system, the available published data were systematized and validated in a workshop with leading regional experts in the field. To identify the technologies for improvement, a survey was conducted among regional farm advisors. Finally, to quantify the impact of adopting improvements in the BASE system, a modelling study was conducted. The results showed that traditional cow-calf systems have low productive and reproductive efficiency (45 kg LW ha^{-1} $year^{-1}$ and 48% weaning rate) and little adoption of herd management and forage production technologies. The technologies identified were grazing management, training of farmers and farm staff, and seasonal mating. The modelling study showed that improvements in the production and use of forage and herd management practices would increase beef production and the gross margin of the BASE system by 70% and 96%, respectively.

Keywords
beef production • survey • technologies • simulation • opportunities

1 Universidad Nacional del Litoral. Facultad de Ciencias Agrarias. R.P. Kreder 2805 2° Piso. C. P. 3080. Esperanza. Argentina. * guillerminagregoretti.gg@gmail.com
2 Universidad Nacional del Litoral. FCA. CONICET. IciAgro Litoral. R. P. Kreder 2805. C. P. 3080. Esperanza. Argentina.
3 Instituto Nacional de Tecnología Agropecuaria. Agencia de Extensión Rural INTA Calchaquí. Bv. Belgrano 939. C. P. 3050. Calchaquí. Argentina.
4 Facultad de Ciencias Veterinarias. Centro de Investigación Veterinaria de Tandil-CIVETAN. CICPBA-CONICET, PROANVET UNCPBA. B7000GHG. Tandil. Argentina.

Resumen

Los sistemas de cría son el núcleo de la importante industria nacional de carne bovina de Argentina. Los objetivos fueron: i) caracterizar la situación productiva de sistemas de cría tradicionales, nombrado BASE, del norte de la provincia de Santa Fe ii) identificar tecnologías para su mejora productiva iii) cuantificar el impacto productivo y económico de la adopción de las tecnologías identificadas. Para caracterizar el sistema BASE se sistematizó la información disponible que fue validada en un taller con expertos referentes de la zona. Para identificar tecnologías de mejora, se implementó una encuesta a asesores referentes de la región. Finalmente, para cuantificar el impacto de la adopción de mejoras en el sistema BASE se realizó un estudio de simulación. Los resultados demostraron que los sistemas de cría tradicionales tienen baja eficiencia productiva y reproductiva (45 kg de PV ha^{-1} año^{-1} y 48% de destete, respectivamente) y baja adopción de tecnologías de manejo del rodeo y producción forrajera. Las tecnologías identificadas fueron manejo del pastoreo, capacitación del productor y el personal de campo y estacionamiento del servicio. La simulación demostró que mejoras en producción y uso de forrajes y manejo de rodeo podrían incrementar la producción de carne y el margen bruto del sistema BASE en un 70% y 96%, respectivamente.

Palabras claves
producción de carne • encuesta • tecnologías • simulación • oportunidades

Introduction

The livestock sector faces the challenge of producing food in the context of increasing global demand for meat, which is estimated to increase by 1.6% per year (30). Intensification has been a way to improve productivity and efficiency in the beef production sector and has contributed to an increase in food production since the mid-twentieth century (18, 38). Intensification of livestock systems is defined as an increase in meat and milk production per animal and per area of land (31). In beef production systems, there are mainly two ways of intensification: through the increase in pasture production and supplementation of animals in grazing systems, or through the confinement of animals in feedlots with high feed offers (20).

Argentina produces 3.1 thousand tonnes of beef and ranks fourth among the world's beef-producing and exporting countries (43). Beef production is a relevant activity for the country's economy because it contributes 28.7% of gross domestic income and 11% of private employment within the agricultural industry (14). However, the national average weaning rate (total of weaned calves/ total of cows × 100) is lower (63%) (26) than that in other beef-producing countries such as Australia (70%) (41) and New Zealand (80%) (42). In Argentina, more than 95% of the area used for cow-calf systems is based on natural grasslands (non-cultivated environments), with poor synchronization between forage supply and livestock nutrient demand, reduced control of animal diseases (15), mainly concerning venereal and reproductive diseases (2), and low stocking rates (less than 0.50 cow ha^{-1}) (26), resulting in low productivity, in terms of beef production per hectare (less than 90 kg ha^{-1}) (26).

Buenos Aires province is the main beef-producing region of Argentina, and different studies have evaluated the impact of technological improvements (3, 16), technical assistance on the productivity of systems (33), and pasture production (22), among others. However, for the second most important region in calves´ provision, the northern region of Santa Fe province, which provides 10% of total Argentine calves (26), there is minimal information regarding the characterization of technified systems (21), but none for traditional systems. Therefore, the objectives of the present study were: i) to characterize the productive situation of the traditional cow-calf systems (hereafter BASE system) in the northern region of Santa Fe province, Argentina; ii) to identify technologies for improving productivity based on critical technologies; and iii) to quantify the productive and economic impacts of applying technologies.

Materials and methods

Description of the region

The cow-calf systems analysed in this study are located in the north-central region of Argentina, between 28° to 30° South and 59° to 60° West in the departments of General Obligado and Vera in the province of Santa Fe. This region has approximately 900,000 ha of agricultural use (10). The climate is subtropical with average, minimum, and maximum annual air temperature of 20.1°C, 10.1°C (July) and 28.2°C (January), respectively (23). The average annual rainfall (±SD) (over the last 50 years) is 1,294 ± 310 mm, concentrated in the warmest season (82% between October and April) (24). Predominant soils belong to the Natracualf and Alfacualf groups, with drainage deficiency and saline-sodium conditions (19).

Productive characterization of the traditional systems

Different sources of information (scientific literature, technical reports, national and regional statistics and a workshop with local experts) were used to characterize the traditional (BASE) system in terms of land use, herd management, forage production, and productive efficiency indicators such as stocking rate (cows ha^{-1}), weaning rate (%), and beef production (kg of calves beef ha^{-1} $year^{-1}$ and kg of LW ha^{-1} $year^{-1}$).

Survey design: Identification and ranking of technologies to improve productivity

A digital survey (Google Forms) was designed to identify and rank technologies that could promote the improvement of traditional systems. The project's interdisciplinary team identified most region-based farm advisors and extensionists with recognized expertise in the field (n = 22) and invited them to complete the survey. The survey was structured into 10 questions. Questions 1 to 6 refer to the degree of agreement that advisors had regarding the priority of improvements in forage resources, herd management, productive and economic records, and farm infrastructure. Questions 7 to 10 refer to the technologies that advisors prioritize to improve forage resources, herd management, and farm infrastructure.

To analyze the results, radar charts were created with the average priority for each option. The prioritization patterns for each question were analyzed using principal components analysis. In addition, the relationship between the prioritizations assigned according to expertise background (agriculture or veterinary science) and work environment (private or public) of the respondents was evaluated using ANOVA. Infostat software version 2018 (12) was used for statistical analyses.

Simulation of productive and economic impact of applying technologies

1- Simulation model: The productive and economic impact of the adoption of the identified technologies in the survey described above was quantified through a participatory modelling approach (17) using Baqueano Cría software (40). This deterministic simulation model represents stabilized cow-calf systems and allows for monthly estimations of herd dynamics, forage and energy balance between feed supply and animal requirements, and productive and economic results. The main inputs of this model include herd composition, prices and live weight of cattle categories, monthly availability of forage, and prices of the main inputs (food, health, and labour). The main outputs included beef production (kg LW ha^{-1} $year^{-1}$) and gross margin (U$S ha^{-1}).

2- Simulation of BASE and improved systems: The traditional cow-calf systems, characterized in the present study (objective i) and named as BASE system, were first simulated. It was used as the baseline to simulate three further scenarios, using technologies to improve productivity and economic results (improved systems) (table 1, page 109). Based on the technologies identified as critical by the experts (objective ii of this study), three improved systems were designed (table 1, page 109): +SR+S, which includes increased stocking rate and supplementation with hay (+39% SR and +173% of hay than the BASE), +EFFICIENCY, which includes higher pregnancy rates and lower mortality rates in cows and calves; and finally +SR+S+E system was simulated, which combined the alternatives +SR+S and +EFFICIENCY. It was assumed that the greater pregnancy efficiency was the result of strategic supplementation (2.5 kg of DM of cottonseed and 1 kg of DM sorghum seed cow^{-1} d^{-1} between May and September) due to its incidence on the body condition of cows (35), and mortality rates were reduced due to better health management, with greater expenses on cow health (+77% compared to BASE).

Table 1. Characteristics of the BASE system and improved systems include: increased stocking rate (+SR), increased reproductive efficiency (+EFFICIENCY) and the combination of both alternatives (+SR+E).

Tabla 1. Características del sistema BASE y sistemas mejorados incluyendo: aumento de carga animal (+SR), aumento de la eficiencia reproductiva (+EFFICIENCY) y la combinación de ambas alternativas (+SR+E).

Variable	Base	+SR+S	+Efficiency	+SR+S+E
Stocking rate, cows/ha	0.30	0.42	0.30	0.42
Pregnancy rate, %	62	62	85	85
Culled cows, %	18	18	5	5
Weaning rate, %	50	50	81	81
Cow mortality rate %	3	3	1	1

3- Productive and economic assumptions: Forage production and utilization for the BASE system were obtained from the database reviewed for objective (i) of this study (table 2), and the same figures were assumed for the improved systems. The mating season was assumed to occur from November to February for all systems.

Table 2. Average forage production (Tn DM ha $^{-1}$ year $^{-1}$) of the traditional cow-calf system of the northern region of Santa Fe province.

Tabla 2. Valores de producción (Tn MS ha^{-1} año^{-1}) de los recursos forrajeros de los sistemas de cría tradicionales del norte de la provincia de Santa Fe.

Environment	Dominant species	Area (ha)	Production (t DM ha $^{-1}$ year $^{-1}$)
Grasslands	*Sorghastrum setosum*	300	5.5
Forest	*Stipa spp.*	210	3.0
Low stratum vegetation	*Leersia hexandra*, *Luziola peruviana*	90	8.3

Economic values are expressed in U.S. dollars (U$S dollars). A cost of US$ 16 cow $^{-1}$ was assumed for animal health. Full-time employees were considered for all farm tasks (180 cows), with a monthly salary of US$744. Herd live weights and farm prices are listed in table 3. The purchase and sale expenses of the different animal categories were 5% and 2% of the price, respectively. The annual gross margin, defined as the difference between net income and direct costs (1), was also simulated, considering the prices of the main products for the region (feed, health, and labour).

Table 3. Herd live weight (kg head $^{-1}$) and farm price (U$S kg $^{-1}$) of different animal categories in a cow-calf system in the northern region of Santa Fe province.

Tabla 3. Peso (kg cabeza^{-1}) y precio (U$S kg^{-1}) de las diferentes categorías en un sistema de cría bovina de la región norte de la provincia de Santa Fe.

Animal class	Live weight (Kg head $^{-1}$)	Price (US$ kg $^{-1}$)
Culling cow	400	1.17
Weaned steer calf	200	2.30
Weaned heifer calf	180	2.09
Heifer	290	1.72
Purchased bulls	900	1.94
Sold bulls	800	0.89

Results and discussion

Productive characterization of the traditional cow-calf system in northern region of Santa Fe

Use of area and forage resources

Three contrasting vegetation environments were differentiated in the region: grasslands, forests, and low-stratum vegetation (27). Such environments are usually found in each farm in proportions of 50%, 35%, and 15% of the total area, respectively (11). The aforementioned diversity of environments poses a challenge for livestock management as they have different herbage mass rates, which implies different grazing management in each environment.

1- Grasslands: It is defined as plant communities dominated by various species where it predominates *Sorghastrum setosum* (Grise.) Hitchc (5, 34). The forage contribution to livestock in these environments varies from 3,000 to 6,000 kg DM ha^{-1}. Other species with high forage value, such as legumes (*i.e.*, genus *Desmodium*, *Desmanthus*, and *Vicia*) and grasses of the genus Paspalum (5), can be found in this environment.

2- Forest: The predominant species in this environment was *Schinopsis balansae* Engl. Plant communities in the forest are dominated by species of the genera *Stipa* and *Piptochaetium* (28). These environments provide forage for cattle in variable quantities and quality (1,000-5,000 kg MS ha^{-1}) according to the state of forest conservation.

3- Low stratum vegetation: These environments are dominated by hygrophilous herbaceous communities dominated by grasses such as *Echinochloa helodes* (Hackel) Parodi, *Leersia hexandra* Sw., and *Luziola peruviana* Juss. Ex J.F. Gmel., with a dry matter production of 6,000 to 8,000 kg ha^{-1} (34).

Improvement of forage production through fertilization or introduction of cultivated species such as perennial pastures or annual forage crops is almost null among the traditional farms in the northern region of Santa Fe province. Cultivated forage species are usually no more than 2% of total area in cow-calf systems some cultivated species are *Avena sativa* L., *Melilotus albus* Medik, *Medicago sativa* L., *Sorghum bicolor* L. Monech and *Chloris gayana* Kunth (6).

Productive and reproductive efficiency and herd management

Mating is continuous throughout the year, with little adoption of herd management and health technologies, such as venereal disease control (13). The age at the first mating is usually greater than 24 months. Supplementation of heifers is carried out occasionally with pasture hay (less than 1 kg DM $animal^{-1}$) in winter and, to a lesser extent, energy concentrates, such as corn and sorghum grains (6). Calve weaning is performed at 8 months of age, the weaning rate is 48%, and beef production is approximately 45 kg LW ha^{-1} $year^{-1}$ (6).

Survey results: opportunities for technological improvement

There was a high level of answers (86% of the invited regional consultants). Respondents were highly experienced experts in veterinary sciences (42%) and agriculture science (58%). The results are presented in table 4 (page 111) and figure 1 (page 112). Priority given to improve herd management was higher for professionals working in the private sector ($p < 0.05$), and in general, answers for each aspect (forage supply, herd management practices, and farm infrastructure) were independent of the career and the field of work of the respondents ($p > 0.05$).

Table 4. Questions 1 to 6 used in the survey to regional farm advisors and answers.
Tabla 4. Preguntas utilizadas en la encuesta a asesores referentes y respuestas.

Questions	Possible answers	Answers
Do better and more precise information on the production and quality of NATURAL forage resources be prioritized for the improvement of cow-calf systems?	Disagree	0.0%
	Little agree	5.3%
	Quite agree	21.1%
	Strongly agree	15.8%
	Totally agree	57.9%
Do better and more information on production and quality of cultivated forage species be a priority for the improvement of cow-calf systems in northern?	Disagree	0.0%
	Little agree	21.1%
	Quite agree	26.3%
	Strongly agree	47.4%
	Totally agree	5.3%
Do herd management (health, seasonal mating, pregnancy diagnosis, and weaning management) be a priority for the improvement of cow calf systems?	Disagree	0.0%
	Little agree	0.0%
	Quite agree	5.3%
	Strongly agree	36.8%
	Totally agree	57.9%
Are productive or economic records priorities for the improvement of cow-calf systems?	Disagree	0.0%
	Little agree	0.0%
	Quite agree	26.3%
	Strongly agree	36.8%
	Totally agree	36.8%
Do you think that farmers make little use of the available records?	Disagree	0.0%
	Little agree	0.0%
	Quite agree	15.8%
	Strongly agree	36.8%
	Totally agree	47.4%
Are infrastructure improvements a priority for the improvement of cow-calf systems?	Disagree	0.0%
	Little agree	5.3%
	Quite agree	47.4%
	Strongly agree	10.5%
	Totally agree	36.8%

Productive and economic impact of technological improvements

The results of the modelling studies are shown in figure 2 (page 112). All three improved systems resulted in higher beef production and a higher gross margin than those of the BASE system. The +SR+S+E alternative showed an increase of 70% and 96% in beef production and gross margin, respectively, compared with the BASE system, despite showing higher direct costs (figure 2, page 112). These results agree with previous simulation studies (16, 17) conducted in other regions of Argentina, which showed that the combination of increased SR increased supplementation, and better reproductive management (similar to +SR+S+E in this study) would increase productive and economic results to a greater extent than if they are implemented as sole alternatives.

A change in stocking rate directly influences income as it correlates with the growth of livestock capital. However, it’s essential to note that the economic efficiency of agricultural systems can be significantly influenced by factors beyond the scope of this study, such as the land tenure regime (39).

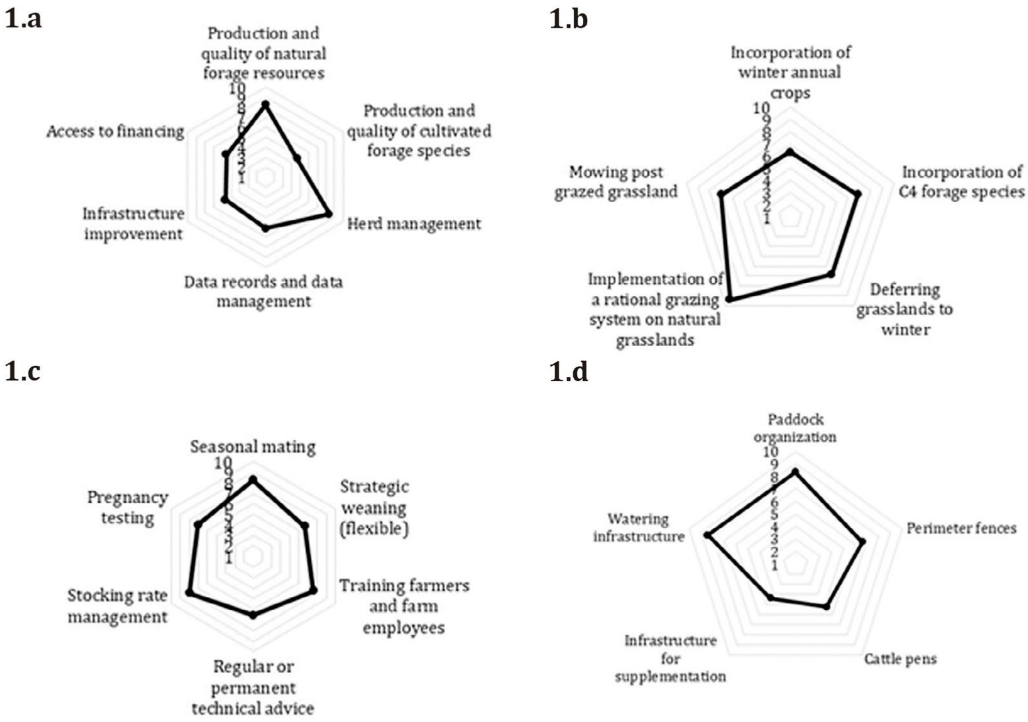


Figure 1. Technologies prioritized by advisors, (10 maximum, 1 minimum). (1.a.) Priority of potential technological improvements.

Figura 1. Tecnologías priorizadas por los asesores, (10 máximo, 1 mínimo). (1.a.) Prioridad de mejoras tecnológicas potenciales.

Priority in: (1.b.) forage supply, (1.c.) herd management, (1.d) infrastructure.

Prioridad en: (1.b.) oferta forrajera, (1.c.) manejo del rodeo, (1.d.) infraestructura.

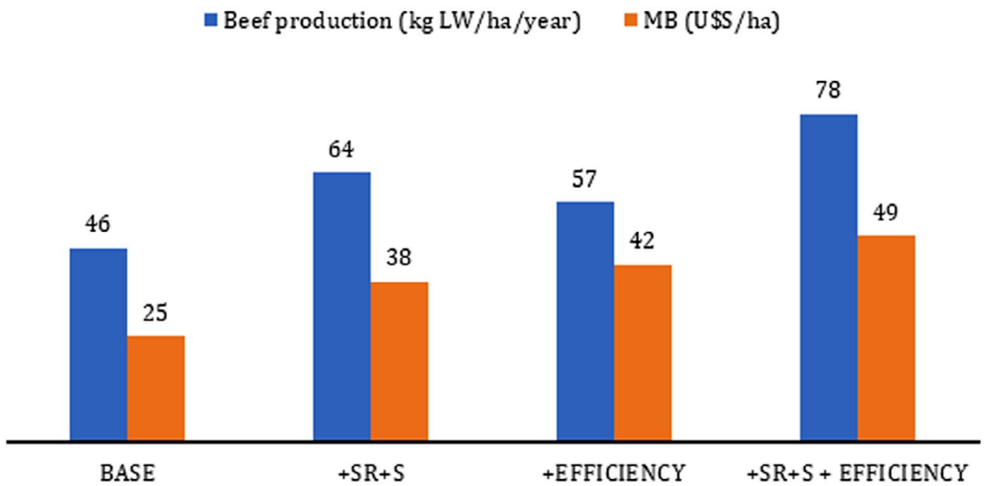


Figure 2. Beef production (kg LW ha^{-1} $year^{-1}$) and gross margin (MB, US\$ ha^{-1} $year^{-1}$) of BASE system and improved alternatives.

Figura 2. Producción de carne (kg PV ha^{-1} $año^{-1}$) y margen bruto (MB, U\$S ha^{-1} $año^{-1}$) del sistema BASE y las alternativas mejoradas.

Table 5 shows previous studies and compares the contrasting productive parameters between the traditional system and existing top technological systems (high use of technologies) in the same region (21). The productive potential of current top cow-calf systems (those having greater technological adoption and management skills compared to traditional farmers in the region) in this region has been recently estimated (21) and the technological gap with the BASE system is 86% in beef productivity (kg/ha/year) and 44% in weaning rate (table 5). This difference is based on the application of technologies that increase forage supply (greater area of cultivated pastures and annual forage crops) and improve herd management techniques, such as greater supplementation of cows, higher stocking rate, seasonal mating, and shorter age for first mating and weaning applied in the top systems compared to the traditional systems.

Table 5. Productive differences between the traditional and top cow-calf systems of the northern region of Santa Fe province.

Tabla 5. Diferencias productivas entre sistemas tradicionales y tecnificados de la región norte de la provincia de Santa Fe.

Productive Variables	Unit	traditional system	Top system (21)
Stocking rate	cows ha^{-1}	0.30	0.46
Weaning rate	%	48	69
Calf beef production	Kg LW ha^{-1} $year^{-1}$	29	51
Beef production	Kg LW ha^{-1} $year^{-1}$	45	83
Grasslands area	% of total area	100	90
Area with cultivated species	% of total area	0	10
Age at first mating	month	27-36	27
Mating strategy		Continuous	Seasonal (4 months)
Weaning age	Months	8	6

Fernandez-Rosso *et al.* (2020) reported 63% more beef production and 340% higher gross margin in systems that combined herd management technologies such as early weaning (2 to 4 months) and implantation of cultivated forage species, in the southwest of Buenos Aires province, compared with traditional systems of that region.

Data available from net aerial primary productivity (NAPP) and the quality of forage available in the region under study are mainly reported for cultivated pastures (32, 36). The productive and economic simulations carried out in this study were based on NAPP data of natural forage resources using a combination of unpublished data of forage cuts validated by experts (table 2, page 109). However, alternative methodologies that allow for the estimation of NAPP have been applied with promising results in other regions of Argentina, such as the green index (22), simulation models (4), and regression equations for forage cuts (17), and could be used in future studies.

In the northern region of Santa Fe Province, there have been several public policies aimed at assisting farmers in improving the productive efficiency of cow-calf systems through subsidized loans and farm advisory support by applying and monitoring health, nutritional, and reproductive management technologies (7, 29). However, the low adoption of technologies and the current low productive and reproductive efficiency (table 5), which have remained stable for years (8, 9), reflect the low effectiveness of those policies. This situation encourages a deeper understanding of the causes of farmers' scarce technological adoption. In other important beef cattle breeding regions of the country, barriers to the adoption of technologies in farming systems are mentioned. In cow-calf system studies located in Buenos Aires province, it has stood out (17, 35) as adoption barriers of technology in the cattle breeding systems of that region due to a lack of training in process technologies, the absence of suitable public policies for the region, and the producers' partial dedication to the activity. Additionally, barriers related to the lack of agricultural vocation among heirs and the absence of technical assistance in low-tech systems have also been described (17).

The applied participatory modelling methodology (17) provided preliminary information and a "what if" analysis (25) of this important productive area. However, the productive characterization of cow-calf traditional systems carried out in this study will require additional research to refine farm information and to define barriers to technological adoption in breeding systems in northern Santa Fe. This understanding might aid in the better design of public policies, which should include the social and cultural conditions of farmers (37). This methodology was also key to the conservation and sustainable development of livestock systems in other countries (44).

Conclusions

We combined the available scarce data on traditional cow-calf systems in the northern region of Santa Fe Province with the qualified knowledge provided by highly experienced farm advisors, in order to establish a benchmark and to identify challenges for future studies. Experts prioritized the improvement of forage supply and herd management to increase the productivity of cow-calf systems. Implementation of a rational grazing system for grasslands, training the farmer and farm staff on herd management, and seasonal mating were the factors selected to be adopted in the first place. The modelling study showed that increased SR, higher supplementation and higher reproductive efficiency increased production and economic results by 70 and 96%, respectively. The participatory modelling methodology applied also allowed us to identify areas in which greater research efforts are needed, such as more precise research information on farm characterisation, forage production and quality, and farmers' constraints for technological adoption, which will be relevant inputs for designing and promoting effective policies for the livestock sector.

References

1. AACREA. 1990. Normas para medir los resultados económicos en las empresas agropecuarias. Convenio AACREA BANCO RIO. Buenos Aires. Argentina.
2. Abdala, A. A.; Maciel, M. G.; Salado, E.; Aleman, R.; Scandolo, D. 2013. Pérdidas de preñez en un rodeo de cría del norte de la provincia de Santa Fe. Rev. Arg. Prod. Anim. 33(2):109- 115.
3. Andreu, M.; Giancola, S. I.; Carranza, A.; Roberi, A.; Serena, J.; Carranza, F.; Nemoz, J. P.; Meyer Paz, R. 2014. Resultados físicos y económicos de la implementación de tecnologías críticas en sistemas ganaderos bovinos de ciclo completo en Cuenca del Salado, provincia de Buenos Aires. Instituto Nacional de Tecnología Agropecuaria, Centro de Investigación en Economía y Prospectiva (CIEP). Azul. Argentina.
4. Berger, H.; Machado, C. F.; Agnusdei, M.; Cullen, B. R. 2014. Use of a biophysical simulation model (DairyMod) to represent tall fescue pasture growth in Argentina. Grass and Forage Science. 69(3): 441-453. doi 10.1111/gfs.12064
5. Capozzolo, M. C.; Crudeli, S. M.; Rollo, L. 2017a. Análisis de la base forrajera de un sistema de cría bovina. Revista Voces y Ecos N° 37. Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Reconquista. Reconquista. Argentina.
6. Capozzolo, C.; Scarel, J.; Ocampo, M. E.; Ybran, R.; Hug, O.; Mitre, P. 2017b. Sistemas ganaderos bovinos - Caracterización del distrito Toba. Instituto Nacional de Tecnología Agropecuaria, EEA Reconquista. Reconquista. Argentina.
7. Cersan. 2006. Proyecto Regional: Producción sustentable de carne bovina en la provincia de Santa Fe. SANFE05. INTA - CERSAN.
8. Chimicz, J. 2006. Tipificación de la Cría bovina en Santa Fe. Una propuesta para la elaboración de estrategias diferenciales de extensión. Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Rafaela, Rafaela. Argentina.
9. Chiossone, G. 2006. Sistemas de producción ganaderos del noreste argentino; Situación actual y propuestas tecnológicas para mejorar su productividad. p. 120-137. En X Seminario de manejo y utilización de pastos y forrajes en sistemas de producción animal. 20-22 de abril. Maracaibo, Venezuela.
10. CNA. 2008. Censo Nacional Agropecuario 2008. https://www.indec.gob.ar/indec/web/Nivel4-Tema-3-8-87. (Date of consultation: 29/03/2023).
11. Dimundo, C. D. 2021. Ciclo completo en ambientes marginales. En El NEA hacia la intensificación ganadera. IPCVA. 24 de febrero. Reconquista, Argentina.
12. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. W. 2011. InfoStat versión 2011. Grupo InfoStat. FCA. Universidad Nacional de Córdoba. Argentina. http:// www.infostat.com.ar. (Date of consultation 29/03/2023).

13. Dolzani, M.; Rosatti, G.; Yaya, A.; Gatti, E.; Bertoli, J.; Zoratti, O.; Ruiz, M.; Podversich, F.; Bressan, E.; Tauber, C. 2019. Causas que limitan la adopción de tecnologías en sistemas de producción de carne del norte de Santa Fe. Argentina. VII Jornada de Difusión de la Investigación y Extensión. Esperanza. Argentina.
14. FADA. 2021. Aporte de las cadenas agroindustriales al PBI. Año 2020. file:///C:/Users/Win10/Downloads/Producto%20Bruto%20Interno%202020.pdf (Date of consultation 29/03/2023).
15. FAO-NZAGRC. 2017. Low-emissions development of the beef cattle sector in Argentina: reducing enteric methane for food security and livelihoods. FAO. Rome.
16. Faverin, C.; Bilotto, F.; Fernández Rosso, C.; Machado, C. F. 2019. Modelación productiva, económica y de gases de efecto invernadero de sistemas típicos de cría bovina de la Pampa Deprimida. Chilean Journal of Agricultural and Animal Sciences. 35(1): 14-25. doi: 10.4067/S0719-38902019005000102.
17. Fernández Rosso, C.; Bilotto, F.; Lauric, A.; De Leo, G. A.; Torres Carbonell, C.; Arroqui, M. A.; Sorensen, C. G.; Machado, C. F. 2020. An innovation path in Argentinean cow–calf operations: Insights from participatory farm system modelling. Systems Research and Behavioral Science. 1-15. doi:10.1002/sres.2679
18. Fuglie, K. O. 2012. Productivity growth and technology capital in the global agricultural economy. In: Fuglie, K. O., Wang, S. L., Ball, V. E. (Eds.). Productivity Growth in Agriculture: An International Perspective. CAB International, Cambridge. p. 335-368.
19. Giorgi, R.; Tosolini, R.; Sapino, V.; Villar, J.; León, C.; Chiavassa, A. 2007. Zonificación agroeconómica de la Provincia de Santa Fe. Delimitación y descripción de las zonas y subzonas agroeconómicas. Publicación Miscelánea N° 110. Instituto Nacional de Tecnología Agropecuaria. Estación Experimental Rafaela. Argentina.
20. Greenwood, P. 2021. Review: An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. Animal: an international journal of animal bioscience. 15(2): 100295. doi 10.1016/j.animal.2021.100295
21. Gregoretti, G.; Baudracco, J.; Dimundo, C.; Alesso, A.; Lazzarini, B. y Machado, C. 2020. Caracterización productiva de sistemas de cría tecnificados de la región centro norte de Argentina. Chilean Journal of Agricultural and Animal Sciences. 36(3): 233-243. doi 10.29393/chjaas36-22cpgg60022.
22. Grigera, G.; Oesterheld, M.; Pacín, F. 2007. Monitoring forage production for farmers' decision making. Agricultural Systems. 94: 637-648. doi 10.1016/j.agsy.2007.01.001
23. INTA. 2018. Estación Meteorológica Reconquista. Instituto Nacional de Tecnología Agropecuaria. https://inta.gob.ar/documentos/estacion-meteorologicareconquista (Date of consultation 29/03/2023).
24. INTA. 2020. Estación Meteorológica Reconquista. Instituto Nacional de Tecnología Agropecuaria. https://inta.gob.ar/documentos/estacion-meteorologica-reconquista (Date of consultation 29/03/2023).
25. Machado, C. F.; Berger, H. 2012. Uso de modelos de simulación para asistir decisiones. en sistemas de producción de carne. Revista Argentina de Producción Animal. 32: 87-105.
26. MAGyP. 2023. Informes Técnicos y Estimaciones. Ministerio de Agricultura Ganadería y Pesca de Argentina. https://www.argentina.gob.ar/agricultura (Date of consultation 29/03/2029).
27. Marino, G.; Pensiero, J. F. 2003. Heterogeneidad florística y estructural de los bosques de *Schinopsis balansae* (Anacardiaceae) en el sur del Chaco Húmedo. Darwiniana 41:17-28. doi 10.14522/darwiniana.2014.411-4.203
28. Martín, S.; Pensiero, J. F.; D´Angelo, C. D. 2006. Bosques para siempre. Las prácticas para un manejo sustentable de los bosques santafesinos. Mesa Agroforestal Santafesina. Argentina.
29. MPSF. 2019. Ministerio de la Producción de Santa Fe. 2019. Programa Más Terneros. Santa Fe. https://www. santafe.gob.ar/index.php/tramites/modul1/index?m=descripcion&imprimir=1&id=245848. (Date of consultation 29/03/2023).
30. OECD-FAO. 2023. Perspectivas agrícolas 2020-2029. https://www.oecd-ilibrary.org/ sites/498ef94e-es/index.html?itemId=/content/component/498ef94e-es#sectiond1e21140. (Date of consultation 29/03/2003).
31. Oenema, O.; De Klein, C. A. M.; Alfaro, A. 2014. Intensification of grassland and forage use: Driving forces and constrains. Crop and Pasture Science. 65(6): 524. doi 10.1071/CP14001
32. Oprandi, G.; Coloombo, F.; Parodi, M.I. 2014. Grama rhodes, una alternativa productiva para los sistemas ganaderos del norte de Santa Fe. Revista Voces y Ecos. 31: 26-27.
33. Pacín, F.; Oesterheld, M. 2015. Closing the technological gap of animal and crop production through technical assistance. Agricultural Systems. 137: 101-107. doi: 10.1016/j.agsy.2015.04.007
34. Pensiero, J. F. 2017. Guía de reconocimiento de herbáceas del Chaco Húmedo. Características para su manejo. Buenas Prácticas para una ganadería sustentable. Fundación Vida Silvestre y Aves Argentinas.
35. Recavarren, P.; Bruno, S.; Torres Carbonell C.; Balda, S.; Kaspar, G. 2021. Resultados del taller "Sistemas de cría vacuna: tecnologías, innovación y extensión en el CeRBAS". Ediciones INTA; Estación Experimental Agropecuaria Balcarce. 16 p.
36. Saucedo M. E.; Castro, C. G.; Obregón, H. J.; Dolzani, E. 2016. Introducción de nuevas pasturas en el norte de Santa Fe. Revista Voces y Ecos. 35: 47-49.

37. Serra, R.; Kiker, G. A.; Minten, B.; Valerio, V. C.; Varijakshapanicker, P.; Wane, A. 2020. Filling knowledge gaps to strengthen livestock policies in low-income countries. Global Food Security. 26: 100428. Doi: 10.1016/j.gfs.2020.100428.
38. Tilman, D.; Cassman, K.; Matson, P.; Naylor, R.; Polasky, S. 2002. Agricultural sustainability and intensive production practices. Nature. 418: 671-677.
39. Troncoso Sepúlveda, R. A.; Cabas Monje, J. H.; Guesmi, B. 2023. Land tenure and cost inefficiency: the case of rice (*Oryza sativa* L.) cultivation in Chile. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 61-75. DOI: https://doi.org/10.48162/rev.39.109
40. Uniagro. 2019. Software Baqueano cría vacuna. www.uniagro.com.ar
41. USDA. 2019a. Livestock and Product Semi-annual. https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Livestock%20and%20Products%20Semiannual_Canberra_Australia_3-1-2019.pdf (Date of consultation 29/03/2023).
42. USDA. 2019b. Cattle and Beef Semi-Annual Report 2019. https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Cattle%20and%20Beef%20Semi-Annual%20Report%202019%20for%20New%20Zealand_Wellington_New%20Zealand_3-12-2019.pdf (Date of consultation 29/03/2023).
43. USDA. 2023. Livestock and Poultry: World Markets and Trade. https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf (Date of consultation 29/03/2023)
44. Vargas-López, S.; Bustamante-González, A.; Ramírez-Bribiesca, J. E.; Torres-Hernández, G.; Larbi, A.; Maldonado-Jáquez, López-Tecpoyotl, Z. G. 2022. Rescue and participatory conservation of Creole goats in the agro-silvopastoral systems of the Mountains of Guerrero, Mexico. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 153-162. DOI: https://doi.org/10.48162/rev.39.074

Acknowledgements

The authors express their gratitude to the Consorcio Regional de Experimentación Agropecuaria Región Norte de Santa Fe (CREA) and its advisors for generously providing the data and collaborating in discussions, offering valuable suggestions for describing the traditional systems in the region. This research is a part of the first author's doctoral studies in Agriculture Science at the Universidad Nacional del Litoral.

This research was funded by a doctoral scholarship from CONICET and a research project from Ministerio de Ciencia y Tecnologia (PICT-2017-2271).

Nutritional quality of amaranth (*Amaranthus*) silage in response to forage airing and addition of lactic bacteria

Calidad nutricional de amaranto (*Amaranthus*) ensilado en respuesta a la aireación del forraje y a la adición de bacterias lácticas

María Fany Zubillaga [1, 2], Julián Agustín Repupilli [1], Patricia Boeri [1,2],
Juan Agustín Servera [1,3], Juan José Gallego [3], Lucrecia Piñuel [1, 2*]

Originales: *Recepción:* 11/05/2023 - *Aceptación:* 29/02/2024

Abstract

Climate change is reducing forage availability for ruminants. Previous studies in Northern Patagonia, Argentina, have demonstrated the adaptation of the amaranth crop to these agroclimatic conditions under irrigation. Moreover, this crop is used as forage in marginal areas of the world, given its outstanding productive and nutritional qualities. The objective of this study was to evaluate the nutritional quality of amaranth silage in response to previous wilting and the addition of lactic acid bacteria. The crop was harvested at the milky grain stage and ensiled in experimental microsilos for 60 days. Before ensiling, different treatments (wilting and addition of lactic acid bacteria) were applied. Parameters related to nutritional quality were evaluated, including ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), dry matter digestibility (DMD), and metabolizable energy (ME). Simultaneous treatment with air and the addition of lactic acid bacteria before ensiling resulted in the best nutritional quality characteristics of the silage. The most significant results were protein value of 12.7%, 41.1% NDF and 19.1% FDA. The DM and ME were 74% and 2.67 Mcal/kg, respectively. Thus, amaranth silage can be considered an alternative conserved forage for animal feed in this region.

Keywords
nutritional value • amaranth • silage • inoculation • quality

1 Universidad Nacional de Río Negro. Sede Atlántica. Don Bosco y Leloir s/n. Viedma (8500). Río Negro. Argentina. * lpinuel@unrn.edu.ar
2 Universidad Nacional de Río Negro (UNRN-CONICET). CIT-RIO NEGRO Sede Atlántica. Don Bosco y Leloir s/n. Viedma (8500). Río Negro. Argentina
3 Instituto Nacional de Tecnología Agropecuaria (INTA). Estación Experimental Valle Inferior de Río Negro. Ruta Nac. N° 3 km 971 - Camino 4 IDEVI (8500) Viedma. Río Negro. Argentina.

Resumen

El cambio climático está reduciendo la disponibilidad de forraje para los rumiantes. Estudios previos en la Patagonia Norte Argentina demuestran la adaptación del cultivo de amaranto a estas condiciones agroclimáticas bajo riego. Sin embargo, este cultivo se utiliza como forraje en zonas marginales del mundo, dadas las destacadas cualidades productivas y nutricionales del cultivo de amaranto. El objetivo de este estudio fue evaluar la calidad nutricional del ensilado de amaranto en respuesta al oreado previo del forraje y a la adición de bacterias lácticas. El cultivo se cosechó en el estado grano lechoso y se ensiló en microsilos experimentales durante 60 días. Antes del ensilado, se llevaron a cabo diferentes tratamientos (oreado y adición de bacterias lácticas). Se evaluaron parámetros relacionados con la calidad nutricional: cenizas, proteína cruda (PC), Fibra Detergente Neutro (FDN), Fibra Detergente Ácido (FDA), digestibilidad de la materia seca (DMS) y energía metabolizable (EM). El tratamiento simultáneo de oreado y adición de bacterias lácticas antes del ensilado dio lugar a las mejores características de calidad nutricional del ensilado obtenido. Los resultados más importantes bajo estas condiciones fueron valores de proteína del 12,7%, FDN del 41,1% y FDA del 19,1%. La DMS y la EM fueron del 74% y 2,67 Mcal/kg, respectivamente. Así, el ensilaje de amaranto puede ser considerado como una alternativa forrajera conservada para la alimentación animal de la región.

Palabras claves
valor nutricional • amaranto • ensilado • inoculación • calidad

Introduction

The projected growth in global food demand has led to an increased focus on underutilized crops, with the potential to improve global food security and mitigate the adverse effects of climate change. Animal production in the Lower Rio Negro Valley is limited by a seasonal lack of forage. Feed and conserved fodder, such as silage, ensures forage quality and quantity. The main silage resource in the area is corn, with a biomass yield of over 30 t/ha and a protein content of 8%. However, its cultivation requires 900 mm of water and 300 kg/ha of nitrogen (27). *Amaranthus cruentus cv* Mexicano has shown adaptability to the local environmental conditions with yields of 21 t/ha, needing 800 mm and 150 kg/ha of water and nitrogen requirements, respectively (39, 40, 41). Therefore, amaranth could be an alternative forage resource for the region given its high biomass production and low management requirements. Seguín *et al.* (2013) stated that amaranth shows high rumen degradability when used as animal feed, either by direct grazing or as conserved forage. The nutritive value of this forage varies according to the developmental stage, standing out for its high crude protein content (8-29%), low lignin values (1.7-7.3%), and high *in vitro* digestibility (59-79%) (23, 28). However, high moisture and protein content could negatively affect the ensiling process. According to Borreani *et al.* (2018), moisture content produces effluents that reduce soluble carbohydrates, whereas high protein values in the forage have a buffering capacity and prevent pH decrease (<5.0) in the silage. A common practice to decrease forage moisture is to wilt the forage in the field for 24-48 hours under good weather conditions (no risk of rain) and with minimal mechanical treatment. This increases dry matter, resulting in a higher concentration of soluble carbohydrates that favors the fermentation process and nutritive value (37). The use of additives, such as bacterial inoculants, could favor a rapid decrease in pH, improving the conservation and nutritive value of silage (4, 32). Among the most commonly used inoculants are lactic acid bacteria (LAB), which ferment carbohydrates into lactic acid, acidifying the silage and inhibiting growth of undesirable bacteria (24), thus improving the fermentation and aerobic stability of the final product. Although silage techniques have been studied for this crop, different results depend on variety, cutting time, processing methodology, and place of origin (16, 20, 28, 29). In South America, there is no information on amaranth as fodder or its conservation in silage. In this study, we hypothesized that wilting and LAB inoculation would improve fermentation quality of amaranth forage. The objective of this study was to evaluate the nutritional quality of amaranth silage in response to previous wilting of forage and addition of lactic acid bacteria.

Materials and methods

Site location, climatic conditions, and forage production

Field experiments were conducted at the INTA-Estación Experimental Agropecuaria Valle Inferior del Río Negro (40°48' S, 63°05' W; 4 m). This area in Patagonia, Argentina, has an irrigation system that covers 24,000 ha. During the growth period of amaranth in 2019 (November to April), rainfall was 186 mm and the average temperature was 19°C. The physicochemical characteristics of the upper 50 cm of the experimental loam soil were: pH (8.20); electrical conductivity (1.2 mmhos cm^{-1}); organic matter (3.8%); total nitrogen (0.18%); N-NO_3 (24.60 mg kg^{-1}); P (Olsen, 16.60 mg kg^{-1}); S (14.7 mg kg^{-1} as a $SO_4^{=}$); Ca (8.230 mg kg^{-1}); Mg (1.170 mg kg^{-1}); sodium-adsorption ratio (1.83). The INTA laboratory performed the soil characterization.

The cultivar evaluated was *A. cruentus* cv Mexicano, sown in rows with a horticultural seeder at the end of spring (November 20th). The sowing area was 70 m^2 (10 furrows 0.7 m wide × 10 m long). Weeding was performed manually when the plants reached 20-30 cm in height; thinning was performed by hand, leaving ten plants m^{-2}. Furrow irrigation was applied according to soil moisture retention curve before reaching the permanent wilting point, with a total lamina of 800±50 mm. Fertilization with granulated urea (46% N), with a nitrogen (N) dose of 90 ha^{-1}, was carried out in two stages; on plants 60 cm high and at bloom.

Treatment of plant material before the silage process

The forage was cut at advanced flowering stage (between milky-pasty grains), corresponding to a chronological time of 123 days or 1627 growing degree days, with dry matter (DM) values above 20%. Amaranth plants were manually cut 50 cm above the soil surface (26) and then wilted in the field for 24 hours before chopping the material. The plants were chopped using a Thomas Willey mill until they reached a size of 1-3 cm. The chopped material was then treated with lactic acid bacteria (LAB) under conditions recommended by the manufacturer using an atomizer. (Bemix Plus® 2% w/v). Four different types of silage were obtained: unwilted, ensiled amaranth (UWAE); unwilted, ensiled amaranth with added lactic acid bacteria (UWAEL); wilted, ensiled amaranth (WAE); and wilted, ensiled amaranth with added lactic acid bacteria (WAEL).

Ensiling process

The silage was prepared at laboratory scale using 110mm PVC tubes 30 cm long, known as experimental microsilage. The filling of the experimental microsilage was performed by placing the plant material in compacted layers with a hydraulic press (140 kgf) ensuring homogeneous compaction. The tubes were capped and sealed to achieve anaerobic conditions (four replicates for each forage type). The experimental microsilage was maintained under environmental conditions for 60 days.

Characterization and chemical analyses of the forage and silage types

Chop size: A fresh sample (100 g) of non-ensiled plant material was measured using a Vernier caliper and grouped according to length. After the ensiling period, the average temperature was determined with an infrared thermometer (AMPROBE IR-710) and a sample (50 g) was collected at a depth of 15 cm from each silo. All samples were frozen at -18°C until use for quality determination. An aqueous extract (1:4) was made and left to rest for 1 h at room temperature, after which pH was measured with a pH meter (Foodcare HI99161) (10). Dry matter content (% DM) was determined according to the AOAC (2000). The samples were ground using a grinder (ARCANO®). Ash content, crude protein (CP%), Neutral Detergent Fiber (NDF %), and Acid Detergent Fiber (ADF %) were determined following the methodology proposed by the AOAC (2000). The dry matter digestibility (% DMD) was estimated using the predictive equation proposed by Rohweder *et al.* (1978). Metabolizable

Energy (ME) was determined using the following formula: EM: 3.61*DMD according to Di Marco (2011). All measurements were performed in triplicate.

Statistical analysis

A completely randomized block design was used, with four treatments and four replicates for each treatment. Pre-ensiling quality variables were analyzed using simple ANOVA (WA-C; UWA-C). Silage quality parameters were analyzed by double ANOVA with the following sources of variation: wilting (UWAE; WAE), inoculation (UWAEL; WAEL), and their interaction as main effects (2 × 2 factorial design). The T° and pH showed no interaction, while DM, CP, ash, NDF, and SDF were analyzed by simple ANOVA to evaluate the effect of wilting and addition of lactic acid bacteria separately. Comparisons of means were made using Fisher's least significant difference (LSD) test at 5%. Statistical analyses were performed using InfoStat (8).

Results and discussion

Pre-ensiling characteristics of forage

Visual differences in coloration between UWA-C and WA-C were observed in the chopped plant material (data not shown). The latter had an opaque yellow-olive color, whereas UWA-C was bright olive-green, which could be associated with the loss of water during the wilting process. In addition, drying plant material in the sun results in the formation of indigestible protein-carbohydrate complexes called Maillard products. The DM values are presented in table 1. Knowing the proper DM content of forage at the pre-ensiling stage is essential to achieve efficient compaction and an anaerobic environment while preventing growth of undesirable microorganisms. DM content of 30-35% is usually recommended to obtain high-quality amaranth silage (14). Krawutschke *et al.* (2013) achieved an optimum DM content of 30-35 % when wilting red clover (*Trifolium pratense L.*), which like amaranth is difficult to ensile due to a lower water-soluble carbohydrate concentration (WSC), higher buffering capacity (BC) and lower dry matter (DM) content at harvest. In amaranth, wilting (WA-C) resulted in dry matter values that reached the recommended values for silage; therefore, wilting could favor a higher concentration of soluble carbohydrates and a lower concentration of lactic acid (21).

Table 1. Nutritional parameters of unwilted amaranth control (UWA-C) and wilted amaranth control (WA-C).

Tabla 1. Parámetros nutricionales del forraje de amaranto no oreado control (UWA-C) y forraje de amaranto oreado control (WA-C).

	DM %	CP %	NDF %	ADF %	DMD %	ME Mcal/kg DM
UWA-C	20.3 b	15.8 a	46.1 b	26.4 b	68.3	2.5
WA-C	34.8 a	14.1 b	51.7a	27.6a	67.4	2.4
Standard error	0.36	0.09	0.58	0.27		
p-value	0.0001	0.0007	0.0001	0.0025		

Dry matter (DM); Crude Protein (CP), Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF), Dry Matter Digestibility (DMD), Metabolizable Energy (ME) associated with the quality of the forage before silage.

Values of the same variable followed by the same letter are not statistically different according to Fisher's LSD test (p > 0.05).

Materia Seca (MS); Proteína Cruda (PC), Fibra Detergente Neutra (FDN); Fibra Detergente Ácida (FA), Digestibilidad de la Materia Seca (DMS), Energía Metabolizable (EM) asociados a la calidad del forraje antes del ensilaje.

Los valores de las mismas variables seguidos de la misma letra no son estadísticamente diferentes según la prueba LSD de Fisher (p > 0,05).

On the other hand, table 1 (page 120), shows the nutritional parameters of the material before the ensiling process and statistically significant differences (p> 0.001) were observed between the UWA-C and WA-C treatments. Forage total N determines the total amount of N available for animal consumption. In this study, the %CP of UWA-C was higher than that of WA-C, possibly because the drying process favors loosing nonstructural carbohydrates, volatile organic substances, and protein degradation. This results in amino acids with higher solubility that can be removed from plant tissues, and although this process can significantly decrease the %CP, it varies depending on the drying method. N decreased by only 5% in this case because of wilting conditions. WA-C had the highest fiber content (NDF and SDF), possibly because exposure of the material to ultraviolet radiation (wilting) decreased forage soluble components. In this study, NDF content ranged from 46.1% to 51.7%, and FAD content ranged from 26.4% to 27% when amaranth plants were wilted. Fiber components, such as NDF, FAD, and lignin, are generally inert during plant respiration, but their concentrations increase because of a decrease in oxidized soluble compounds. Therefore, slow-drying methods are expected to result in higher proportions of NDF and FAD. The digestibility of UWA-C was significantly higher (p> 0.001) than that of WA-C, mainly given by the lower fiber content of the undried plant material.

On the other hand, the size of the plant material obtained in this study before ensiling was between adequate ranges (1-3 cm), coinciding with the recommendations of Citlak and Kilic (2020).

Determination of parameters related to fodder processing and conservation

A double ANOVA determined that the wilting and inoculation treatments did not affect the temperature and pH variables. However, as shown in table 2, significant differences in T° were observed with the wilting effect, where the minimum and maximum values (UWAE and WAE, respectively) were recorded, but there were no differences in this parameter after LAB addition. Temperature affected silage fermentation, and the best results were obtained with a moderate T between 20 and 30°C. For corn silage, Zhou *et al.* (2019), reported that increasing storage temperature from 5°C to 25°C did not affect fermentation profiles of most biochemical parameters or bacterial and fungal populations. In the present study, the low temperatures observed when opening the experimental microsilage could be related to low ambient temperature at the study site.

Table 2. Parameters related to fodder processing and conservation.

Tabla 2. Parámetros relacionados con el procesado y conservación del forraje.

Treatment	T° (°C)	pH
UWAE	15.95 ± 1.42 b	4.6 ± 0.13b
WAE	18.10 ± 1.17 a	4.9 ±0.22a
UWAEL	16.05 ± 1.86 ab	4.5 ± 0.04b
WAEL	17.50 ± 0.71 ab	4.6 ± 0.71b
Standard error	0.68	0.05
p-valor	0.6158	0.0595

Temperature (T°), and pH of the experimental microsilage at the moment of opening for unwilted amaranth ensiled (UWAE); unwilted amaranth ensiled with added lactic acid bacteria (UWAEL); wilted amaranth ensiled (WAE); wilted amaranth ensiled with added lactic acid bacteria (WAEL).

Values of the same variable followed by the same letter are not statistically different according to Fisher's LSD test (p > 0.05).

Temperatura (T°), y pH de los microsilos experimentales en el momento de apertura. Amaranto ensilado sin orear (UWAE); amaranto ensilado oreado (WAE); amaranto ensilado sin orear con adición de bacterias lácticas (UWAEL); amaranto ensilado oreado con adición de bacterias lácticas (WAEL).

Los valores de las mismas variables seguidos de la misma letra no son estadísticamente diferentes según la prueba LSD de Fisher (p > 0,05).

When anaerobic fermentation occurs in the silage process, lactic acid bacteria in the forage are desirable because they rapidly lower the pH, achieve rapid stabilization, and maintain the characteristics of the ensiled material (9, 36). The pH (average of 4.65) observed for all silages was within the established values for good-quality silages, which is comparable with the values reported for different amaranth varieties, harvest stages, and place of cultivation (26). Several factors may be responsible for silage having a higher than normal pH (~4.0), including buffering capacity provided by high protein content (17). In UWAEL and WAEL, the use of bacterial inoculants slightly increased acidity of the experimental microsilage. Similar results have been obtained for corn, oats, and amaranth silages (4, 20). In alfalfa, a forage with high buffering capacity, wilting pretreatment, and a pH of 5.19 was reported after 60 days of ensiling (11). In this study, silage with pre-wilting and higher DM (WA-C, table 1, page 120) showed a slight increase in T° and pH; however, the silage obtained with wilted forage and addition of lactic acid bacteria (WAEL) only showed a decrease in pH.

Other authors have reported that lactic acid bacteria in temperate to cold environments favor a lower pH and rapid production of desirable metabolites, such as lactic acid, accelerating fermentation, and better-conserving silage nutrients over a wide range of growth temperatures (4, 13).

Nutritional quality of ensiled plant material

For the DM, CP, Ash, NDF, and ADF variables, the double ANOVA detected interactions between sources of variation (wilting and inoculation with LAB); therefore, the results are presented separately (table 3). When DM was determined after the ensiling process, values of approximately 20% were observed in the unwilted plant material (UWAE and UWAEL) and values approached 34% in the wilted material (WAE and WAEL). These values are similar to those obtained before ensiling (table 1, page 120). Good fermentation results in DM losses of less than 10% (3), and the dry matter reduction was <1% in our case.

Table 3. Parameters related to nutritional quality.

Tabla 3. Parámetros relacionados con la calidad nutricional.

Without LAB	**DM (%)**	**CP (%)**	**ASH (%)**	**NDF (%)**	**ADF (%)**	**DMD (%)**	**ME (Mcal/kg DM)**
UWAE	20.24b	11.14b	14.19a	48.58a	26.36a	68.37	2.47
WAE	34.47a	13.43a	14.31a	44.07b	24.30b	69.97	2.53
Standard error	0.58	0.12	0.01	0.06	0.03		
p-value	0.0001	0.0001	0.0603	0.0001	0.0001		
With LAB	**DM (%)**	**CP (%)**	**ASH (%)**	**NDF (%)**	**ADF (%)**	**DMD (%)**	**ME (Mcal/kg DM)**
UWAEL	20.66b	13.32a	15.23a	48.80a	26.61a	68.17	2.46
WAEL	33.59a	12.71b	12.87b	41.11b	19.12b	74	2.67
Standard error	0.25	0.56	0.03	0.04	0.28		
p-value	0.0001	0.0299	0.0001	0.0001	0.0001		

Dry Matter (DM); Crude Protein (CP), Neutral Detergent Fiber (NDF); Acid Detergent Fiber (ADF), Digestibility of Dry Matter (DMD), Metabolizable Energy (ME) of ensiled plant material. Unwilted amaranth ensiled (UWAE); wilted amaranth ensiled (WAE); unwilted amaranth ensiled with added lactic acid bacteria (UWAEL); wilted amaranth ensiled with added lactic acid bacteria (WAEL).

Values of the same variable followed by the same letter are not statistically different according to Fisher's LSD test (p > 0.05).

Materia Seca (MS); Proteína Cruda (PC), Fibra Detergente Neutra (FDN); Fibra Detergente Ácida (FDA), Digestibilidad de la Materia Seca (DMS), Energía Metabolizable (EM)) del material vegetal ensilado. Ensilado de amaranto sin orear (UWAE); ensilado de amaranto oreado (WAE); ensilado de amaranto sin orear con adición de bacterias lácticas (UWAEL); ensilado de amaranto oreado con adición de bacterias lácticas (WAEL).

Los valores de las mismas variables seguidos de la misma letra no son estadísticamente diferentes según la prueba LSD de Fisher (p > 0,05).

High protein content is generally associated with higher forage quality, and protein content has been reported to vary from 11.5 to 14% in amaranth silage (16). These variations could be associated with on-site environmental conditions and agronomic practices affecting nutritional composition (39). Table 3 (page 122) shows protein content of the different silages used in this study, with values within the mentioned range. Thus, UWAE had the lowest value (11.14%) for this variable, whereas WAE had the highest value (13.43%). A decrease in protein content of the ensiled material was observed when compared with the non-silage forage (WA-C and UWA-C). The greatest decrease in protein content was observed in UWAE (26% and 12% in UWAE and UWAEL, respectively); however, these decreases were smaller in previously wilted forage (7% WAE and 10% WAEL). In general, a higher osmotic pressure results from higher dry matter (DM) content, which affects plant enzymatic activity and reduces proteolytic capacity (16, 30). This leads to a decrease in soluble protein (CP) and an increase in the insoluble protein fraction, although the latter is still potentially degradable in wilted silages (34, 35).

In contrast, the addition of LAB to unwilted forage decreased proteolysis by 54% and CP loss decreased. However, in the case of WAEL, the addition of LAB did not decrease proteolysis, indicating an interaction between wilting and the addition of LAB, reflected in a decrease in CP. These results agree with those published by Abbasi *et al.* (2018), who observed decreases in protein content in relation to fresh material of 9%, 10%, and 7.5% in ensiled forage, silage with lactic acid bacteria, and wilted forage, respectively.

Ash contents of the different experimental microsilages is listed in table 3 (page 122). The silages with BL (UWAEL and WAEL) had the highest and lowest ash values, respectively. However, silages without the addition of lactic acid bacteria (UWAE-WAE) did not show statistically significant differences ($p>0.05$), and the wilting process did not affect ash content. Amaranth species are characterized by high mineral content that can vary according to variety, harvest time, climate, soil, and crop management (39). The ash values obtained were within the range of those published by other authors for amaranth silage, which provides more minerals for livestock (25, 28). Seguín *et al.* (2013) reported that the major mineral in amaranth is Ca, generally found as calcium carbonate, and a fermentation process can favor the forage buffering capacity to the detriment of its acidification.

Fibers in a feed consist of structural carbohydrates in the cell walls and soluble or nonstructural carbohydrates. Concerning fiber content of fresh chopped forages (UWA-C and WA-C), the ensiling process reduced the NDF and ADF values, in agreement with previous reports (16, 20, 25). Fiber values in the silages obtained (table 3, page 122) were within the ranges described in the literature for this crop (NDF: 28-47.7% and ADF: 26-31%) (14, 21). ADF and NDF showed statistically significant differences due to the effect of wilting, and a decrease was observed as a consequence. However, the addition of LAB further favored this decrease in ADF and NDF. Similar results have been reported for amaranth under similar conditions (wilting and addition of LAB) (1). However, in our study on unwilted silages, no such decrease was observed. The higher moisture content and lower temperature of the experimental microsilage in UWAE and UWAEL may have limited the fermentation process and decreased the action of cellulolytic bacteria responsible for the reduction in the fibrous fraction (14, 38). Fiber content is directly related to digestibility and influences the rate at which feed passes through the digestive tract of an animal (18). Digestibility values for UWAE and UWAEL mirrored those observed for FDN and FAD, and the addition of LAB did not increase this parameter. However, WAE and WAEL showed higher digestibility and metabolizable energy values (table 3, page 122). A synergistic effect was observed between wilting and the addition of LAB, achieving silage with a digestibility of 74.02%. This silage would be classified as good quality according to Di Marco's classification (7), whereas the rest of the silages would be of medium quality. Regarding the ME of WAEL (2.67 Mcal/k DM), an 8% increase was observed with respect to the non-silage plant material (WA-C). This energy value can be expressed as 11.17 MJ/k DM and is within the range determined for silages of other amaranth species (1, 29). Based on the results obtained in this study, we can conclude that WAEL presented the best organoleptic, conservation, and nutritional characteristics among the four silages evaluated. By comparing the quality of this silage with the values described for crops traditionally used in this type of conservation technique (table 4, page 124), we can infer that it is comparable to the quality of corn silage.

Table 4. Comparison of nutritional quality parameters of the main crops used as fodder.
Tabla 4. Comparación de los parámetros de calidad nutricional de los principales cultivos utilizados como forraje.

	DM (%)	pH	CP (%)	Ash (%)	ADF (%)	NDF (%)	DMD (%)	ME	Reference
Corn	36	3.5	8.5	5.5	15.8	36.6	75.5	2.7	(21)
Sorghum	33.7	4	6.9	8.7	30.7	55	66.3	2.4	(6)
Amaranth	33.5	4.6	12.7	12.9	19.1	41.1	74	2.7	This work

Dry Matter (DM); pH; Crude Protein (CP), Ashes; Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF); Digestibility of Dry Matter (DMD), Metabolizable Energy (ME).

Materia seca (MS); pH; Proteína Cruda (PC), Cenizas; Fibra Detergente Ácida (FDA); Fibra Detergente Neutra (FDN); Digestibilidad de la Materia Seca (DMS), Energía Metabolizable (EM).

Likewise, it is evident that amaranth protein content did not affect the ensiling process, and its digestibility was comparable to that of corn silage. Regarding corn biomass production for silage, previously reported values in the Lower Rio Negro Valley vary between 16-34 Tn DM/ha, depending on the hybrid and management (19). In this sense, amaranth yields (7.8-21 Tn DM/ha) were comparable to those of corn (39).

Conclusion

Wilting and inoculation of amaranth forage with lactic acid bacteria before ensiling resulted in silage with nutritional characteristics (crude protein percentage, fiber content, dry matter digestibility, and metabolizable energy) that can be classified as a high-quality component of animal diets. The practice of ensiling amaranth as an alternative for conserving forage can be considered viable in semi-arid regions such as Patagonia. However, further research under *in vivo* conditions is required, especially regarding animal responses according to category and species, as well as the exploration of combinations with other ingredients to achieve complementarity and a better balance of nutrients and energy.

References

1. Abbasi, M.; Rouzbehan, Y.; Rezaei, J.; Jacobsen, S. E. 2018. The effect of lactic acid bacteria inoculation, molasses, or wilting on the fermentation quality and nutritive value of amaranth (*Amaranthus hypochondriaus*) silage. Journal of animal science. 96(9): 3983-3992. https://doi.org/10.1093/jas/sky257.
2. AOAC. Official Methods of Analysis. 2000. 17th ed. The Association of Official Analytical Chemists Gaithersburg MD USA.
3. Borreani, G.; Tabacco, E.; Schmidt, R. J.; Holmes, B. J.; Muck, R. E. 2018. Silage review: Factors affecting dry matter and quality losses in silages. Journal of Dairy Science. 101(5): 3952-3979.
4. Chen, L.; Bai, S.; You, M.; Xiao, B.; Li, P.; Cai, Y. 2020. Effect of a low temperature tolerant lactic acid bacteria inoculant on the fermentation quality and bacterial community of oat round bale silage. Animal Feed Science and Technology. 269: 114669. https://doi.org/10.1016/j.anifeedsci.2020.114669.
5. Citlak, H.; Kilic, U. 2020. Innovative Approaches in Covering Materials Used in Silage Making. International Multilingual. Journal of Science and Technology. 5: 2046-2050. http://www.imjst.org/wp-content/uploads/2020/12/IMJSTP29120390.pdf
6. De León, M.; Giménez, R. A. 2011. Ensilajes de sorgo y maíz: rendimiento, composición, valor nutritivo y respuesta animal. https://inta. gob. ar/sites/default/files/ scripttmp-ensilajes_de_sorgo_y_maz_rendimiento_composicin_va. pdf (February 2018).
7. Di Marco, O. 2011. Estimación de calidad de los forrajes. Producir XXI. 20(240): 24-30. https://www.produccion-animal.com.ar/tablas_composicion_alimentos/45-calidad.pdf
8. Di Rienzo, J. A. InfoStat versión 2020. UNC Argentina.

9. dos Santos, A. P. M; Santos, E. M.; Silva de Oliveira, J.; Garcia Leal de Araújo, G.; de Moura Zanine, A.; Araújo Pinho, R. M.; Costa do Nascimento, T. V.; Fernandes Perazzo, A.; Ferreira, D. de J.; da Silva Macedo, A. J.; de Sousa Santos, F. N. 2023. PCR identification of lactic acid bacteria populations in corn silage inoculated withlyophilised or activated Lactobacillus buchneri. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(1): 115-125. DOI: https://doi.org/10.48162/rev.39.101
10. Faithfull, N. T. 2002. Methods in Agricultural Chemical Analysis: A Practical Handbook. CAB International. 304 p.
11. Huo, W.; Zhang, Y.; Zhang, L.; Shen, C.; Chen, L.; Liu, Q.; Guo, G. 2022. Effect of lactobacilli inoculation on protein and carbohydrate fractions, ensiling characteristics and bacterial community of alfalfa silage. Frontiers in Microbiology. 13: 1070175.
12. Krawutschke, M.; Thaysen, J.; Weiher, N.; Taube, F.; Gierus, M. 2013. Effects of inoculants and wilting on silage fermentation and nutritive characteristics of red clover grass mixtures. Grass and Forage Science. 68: 326-338. https://doi.org/10.1111/j.1365-2494.2012.00892.x
13. Kung, J. L.; Shaver, R. D.; Grant, R. J.; Schmidt, R. J. 2018. Silage review Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science. 101: 4020-4033. https://doi.org/10.3168/jds.2017-13909
14. Liu, Q.; Zong, C.; Dong, Z.; Wu, J.; Zhu, J.; Li, J.; Shao, T. 2020. Effects of cellulolytic lactic acid bacteria on the lignocellulose degradation, sugar profile and lactic acid fermentation of highmoisture alfalfa ensiled in low-temperature seasons. Cellulose. 27: 7955-7965. https://doi.org/10.1007/s10570-020-03350-z
15. Liu, Y. F.; Qiu, H. R.; Yu, X.; Sun, G. Q.; Ma, J.; Zhang, D. L.; Senbati, H. 2017. Effects of addition of lactic acid bacteria, glucose, and formic acid on the quality of *Amaranthus hypochondriacus* silage. Acta Prataculturae Sinica. 26: 214-220. DOI: 10.11686/cyxb2017164
16. Lotfi, S.; Rouzbehan, Y.; Fazaeli, H.; Feyzbakhsh, M. T.; Rezaei, J. 2022. The Nutritional Value and Yields of Amaranth (*Amaranthus hypochondriacus*) Cultivar Silages Compared to Silage from Corn (*Zea mays*) Harvested at the Milk Stage Grown in a Hot-humid Climate. Animal Feed Science and Technology. 289: 115336. https://doi.org/10.1016/j.anifeedsci.2022.115336
17. McDonald, P.; Henderson, A. R.; Heron, S. J. E. 1991. The biochemistry of silage. Chalcombe publications.
18. Mertens, D. R.; Grant, R. J. 2020. Digestibility and intake. Forages: the science of grassland agriculture. 2: 609-631. https://doi.org/10.1002/9781119436669.ch34
19. Miñon, D. P.; Gallego, J. J.; Barbarossa, R. A.; Margiotta, F. A.; Martinez, R. S.; Reinoso, L. 2009. Evaluación de la producción de forraje de híbridos de maíz para silaje en el Valle Inferior del Río Negro (campaña 2008-2009). INTA EEA Valle Inferior.
20. Mu, L.; Xie, Z.; Hu, L.; Chen, G.; Zhang, Z. 2021. *Lactobacillus plantarum* and molasses alter dynamic chemical composition, microbial community, and aerobic stability of mixed (amaranth and rice straw) silage. Journal of the Science of Food and Agriculture. 101: 5225-5235. https://doi.org/10.1002/jsfa.11171
21. Patterson, J. D.; Sahle, B.; Gordon, A. W.; Archer, J. E.; Yan, T.; Grant, N.; Ferris, C. P. 2021. Grass silage composition and nutritive value on Northern Ireland farms between 1998 and 2017. Grass Forage Science. 76: 300-308. https://doi.org/10.1111/gfs.12534
22. Pineda, J. A.; Sánchez, M. E.; Scaramuzza, J. P. 2015. Estudio comparativo de calidad y valor nutritivo de silos bolsa de maíz en la zona de James Craik-Córdoba (Bachelor's thesis).
23. Pisarikova B.; Peterka, J.; Trckova, M.; Moudry, J.; Zraly, Z.; Herzig, I. 2007. The content of insoluble fibre and crude protein value of the aboveground biomass of *Amaranthus cruentus* and *A. hypochondriacus*. Czech Journal of Animal Science. 52: 348-353. DOI:10.17221/2339-CJAS
24. Queiroz, O. C. M.; Arriola, K. G.; Daniel, J. L. P.; Adesogan, A. T. 2013. Effects of 8 chemical and bacterial additives on the quality of corn silage. Journal of Dairy Science. 96(9): 5836-5843.
25. Rahjerdi, N. K.; Rouzbehan, Y.; Fazaeli, H.; Rezaei, J. 2015 Chemical composition, fermentation characteristics, digestibility, and degradability of silages from two amaranth varieties (Kharkovskiy and Sem), corn, and an amaranth-corn combination. Journal of Animal Science. 93: 5781-5790. https://doi.org/10.2527/jas.2015-9494
26. Ramírez Ordóñes, S.; Rueda, J. A.; Medel Contreras, C. I.; Hernández Bautista, J.; Corral Luna, A.; Portillo, M. F. 2023. Effect of cutting height, a bacterial inoculant and a fibrolytic enzyme on corn (*Zea mays* L.) silage quality. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 129-140. DOI: https://doi.org/10.48162/rev.39.115
27. Reinoso, L. G.; Martinez, R. S.; Otegui, M. E.; Mercau, J.; Gutierrez, M. 2018. Rendimiento potencial de maíz en los valles de Norpatagonia: una aproximación desde los modelos de simulación.
28. Rezaei, J.; Rouzbehan, Y.; Fazaeli, H. 2009 Nutritive value of fresh and ensiled amaranth (*Amaranthus hypochondriacus*) treated with different levels of molasses. Animal Feed Science and Technology. 151: 153-160. https://doi.org/10.1016/j.anifeedsci.2008.12.001
29. Rezaei, J.; Rouzbehan, Y.; Fazaeli, H.; Zahedifar, M. 2014. Effects of substituting amaranth silage for corn silage on intake, growth performance, diet digestibility, microbial protein, nitrogen retention and ruminal fermentation in fattening lambs. Animal Feed Science and Technology. 192: 29-38. https://doi.org/10.1016/j.anifeedsci.2014.03.005

30. Rohweder, D.; Barnes, R. F.; Jorgensen, N. 1978. Proposed hay grading standards based on laboratory analyses for evaluating quality. Journal of Animal Science. 47: 747-759. https://doi.org/10.2527/jas1978.473747x
31. Seguin, P.; Mustafa, A. F.; Donnelly, D. J.; Gélinas, B. 2013. Chemical composition and ruminal nutrient degradability of fresh and ensiled amaranth forage. Journal of the Science of Food and Agriculture. 93(15): 3730-3736.
32. Silveira Pimentel, P. R.; dos Santos Brant, L. M.; Vasconcelos de Oliveira Lima, A. G.; Costa Cotrim, D.; Costa Nascimento, T. V.; Lopes Oliveira, R. 2022. How can nutritional additives modify ruminant nutrition? Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 175-189. DOI: https://doi.org/10.48162/rev.39.076
33. Slottner, D.; Bertilsson, J. 2006. Effect of ensiling technology on protein degradation during ensilage. Animal feed science and technology. 127(1-2): 101-111.
34. Tamminga, S.; Ketelaar, R.; Van Vuuren, A. M. 1991. Degradation of nitrogenous compounds in conserved forages in the rumen of dairy cows. Grass and Forage Science. 46(4): 427-435.
35. Verbič, J.; Ørskov, E. R.; Žgajnar, J.; Chen, X. B.; Žnidaršič-Pongrac, V. 1999. The effect of method of forage preservation on the protein degradability and microbial protein synthesis in the rumen. Animal feed science and technology. 82(3-4): 195-212.
36. Villa, R.; Rodriguez, L. O.; Fenech, C.; Anika, O. C. 2020. Ensiling for anaerobic digestion: A review of key considerations to maximise methane yields. Renewable and Sustainable Energy Reviews. 134: 110401. https://doi.org/10.1016/j.rser.2020.110401
37. Wan, J. C.; Xie, K. Y.; Wang, Y. X.; Liu, L.; Yu, Z.; Wang, B. 2021. Effects of wilting and additives on the ensiling quality and *in vitro* rumen fermentation characteristics of sudangrass silage. Animal Bioscience. 34(1): 56. DOI: 10.5713/ajas.20.0079
38. Zhou, Y.; Drouin, P.; Lafrenière, C. 2019. Effects on microbial diversity of fermentation temperature (10°C and 20°C), long-term storage at 5°C, and subsequent warming of corn silage. Asian-Australasian Journal of Animal Sciences. 32: 1528. DOI:10.5713/ajas.18.0792
39. Zubillaga, M. F.; Camina, R.; Orioli, G. A.; Barrio, D. A. 2019. Response of *Amaranthus cruentus* cv Mexicano to nitrogen fertilization under irrigation in the temperate, semiarid climate of North Patagonia, Argentina. Journal of plant nutrition. 42: 99-110. https://doi.org/10.1080/01904167.2018.1549674
40. Zubillaga, M. F.; Camina, R.; Orioli, G.; Failla, M.; Barrio, D. A. 2020. Amaranth in southernmost latitudes: plant density under irrigation in Patagonia, Argentina. Revista Ceres. 67: 93-99. https://doi.org/10.1590/0034-737X202067020001
41. Zubillaga, M. F.; Martínez, R. S.; Camina, R.; Orioli, G. A.; Failla, M.; Alder, M.; Barrio, D. A. 2021. Amaranth irrigation frequency in northeast Patagonia, Argentina. Biotechnologie, Agronomie, Société et Environnement. 25: 247-258. DOI: 10.25518/1780-4507.19310

Acknowledgements, financial support and full disclosure

Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Río Negro is gratefully acknowledged. The authors thank to INTA Valle Inferior del Río Negro for the possibility to conduct this study.

Authors assures there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

Morphostructural composition and meat quality in local goat kids from the northeastern region of Mexico

Composición morfoestructural y calidad de la carne en cabritos locales de la región noreste de México

Yuridia Bautista-Martínez [1], Lorenzo Danilo Granados-Rivera [2], Rafael Jimenez-Ocampo [3], Jorge A. Maldonado-Jáquez [4*]

Originales: *Recepción:* 26/06/2023 - *Aceptación:* 22/03/2024

Abstract

Goat farming is an important activity in northern Mexico. In this sense, "cabrito" or kid goat is a typical regional dish with high economic and cultural value. However, information on the morphostructural composition and meat quality of these local specimens is scarce. Given this, the objective was to evaluate morphostructural characteristics, carcass and meat quality in local kids according to sex in the northeastern region of Mexico. For this purpose, 14 kids (7 males and 7 females) 57 days old were slaughtered. Morphostructural composition was evaluated with 22 zoomometric and phenotypic variables. Carcass characteristics were evaluated by considering different body structures, carcass yield and degree of fatness. Meat quality was determined by physicochemical characteristics, nutritional value and fatty acid profile. The sex effect was evaluated by t-test of independent means and Chi-square. Meat physicochemical characteristics, nutritional value and morphostructure of local kids were heterogeneous and showed no differences (P≥0.05) concerning sex. Carcass, kidneys, head, neck, rib and loin weights were higher in males than in females (P≤0.05). Fatty acids (FA) found in greater proportion were palmitic (C16:0), oleic (C18:1, n-9), stearic (C18:0), and myristic (C14:0). These FA comprised 80.85 % of the lipid profile of male meat and 76.83% of females. These results are the basis for future programs aimed to improve production systems. Differences found could shed light on future efforts on how to differentiate goat meat from this region of Mexico and enter new markets directly benefiting small producers.

Keywords
meat • carcass • nutritional quality • fatty acid profile

1 Universidad Autónoma de Tamaulipas. Facultad de Medicina Veterinaria y Zootecnia. 87000. Ciudad Victoria, Tamaulipas. México.
2 Instituto Nacional de Investigaciones Forestales, Agricolas y Pecuarias. Campo Experimental General Terán. 67400. General Terán, Nuevo León. México.
3 Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Valle del Guadiana. 34170. Durango, Durango. México.
4 Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental La Laguna. 27440. Matamoros. Coahuila. México.* maldonadoj.jorge@hotmail.com

Resumen

La caprinocultura es una actividad muy importante en el norte de México por la producción de leche y carne. En este sentido, el cabrito es un platillo típico de esta región con valor económico y cultural elevado, no obstante, la información que describe la composición morfoestructural y calidad de carne de estos ejemplares locales, ha sido poco documentada. Debido a lo anterior, el objetivo fue evaluar las características morfoestructurales, calidad de canal y carne en cabritos locales de acuerdo con el sexo (machos y hembras) en la región noreste de México, para esto se sacrificaron 14 cabritos (7 machos y 7 hembras) de 57 días de edad. Para evaluar la composición morfoestructural, se consideraron 22 variables zoométricas y fenotípicas. Las características de la canal se evaluaron considerando las distintas estructuras corporales, rendimiento en canal y grado de engrasamiento. Se determinó calidad de carne midiendo las características fisicoquímicas, valor nutricional y perfil de ácidos grasos. Se evaluó el efecto del sexo mediante una prueba de t de medias independientes y Chi cuadrada. La estructura morfoestructural de los cabritos locales es heterogénea, y no mostraron diferencias ($P \geq 0,05$) respecto del sexo. El peso de la canal, riñones, cabeza, cuello, costillar y lomo fueron mayores en machos que en hembras ($P \leq 0,05$). Las características fisicoquímicas y valor nutricional de la carne no mostraron diferencias entre sexos. Los ácidos grasos (AG) que se encontraron en mayor proporción fueron; palmítico (C16:0), oleico (C18:1, n-9), esteárico (C18:0), y mirístico (C14:0). Estos AG comprendieron el 80,85 % del perfil lipídico de la carne de los machos, mientras que en hembras representaron el 76,83 %. Estos resultados son la base para futuros programas de mejora del sistema productivo y donde las diferencias encontradas podrían arrojar luz sobre esfuerzos futuros, sobre cómo diferenciar la carne de cabrito en esta región de México e ingresar a nuevos mercados que beneficiarían directamente a los pequeños productores.

Palabras clave

carne • canal • calidad nutricional • perfil de ácidos grasos

Introduction

In Mexico, goat production is focused on meat and milk. During 2022, carcass meat production achieved 77,000 tons, and 160 million liters of milk (32). The predominant production system is extensive, with animals known as “local”, with an undefined phenotype derived from mating different breeds such as Alpina, Saanen, Nubia, and Toggenburg (31). Besides feeding suckling kids, the produced milk is used for cheese, cajeta (a milk candy-type), and typical regional candies. Kids are slaughtered and consumed at approximately 30 days old. Their meat is soft and tender, low-fat, pearly white and juicy. As a traditional Mexican dish, particularly in the north of Mexico, it is consumed on special events and can be cooked in various presentations like “al pastor” (shawarma), fried, or roasted. It is also considered a gourmet dish reaching high prices in restaurants (32).

Similar dishes are prepared in other countries such as Spain, where this meat should meet certain characteristics (9), India, China, Pakistan, Nigeria, Bangladesh, and Iran, where goat production is important (13). However, in Mexico, particularly in the northeast, information on body structure, carcass quality, and nutritional properties of kid meat is scarce. Nevertheless, breeds such as Payoya, Gokcead, Maltese, Majorera, Blanca celtiberica, Negra serrana and Moncaica, have been extensively documented (6).

In this context, generating local information on kid meat produced in this region of Mexico is important given that it supplies almost all the consumed kid meat in the northern states of Mexico. Regional goat raising relies on the environmentally best-adapted breeds. In this sense, particular production strategies can set particular qualities, where denominations of origin can trigger added value (26). The aforementioned is particularly important for small-scale, and highly social and economically marginalized producers (31, 35). Therefore, our objective was to evaluate morphostructural traits, carcass and meat quality, and fatty acid profile considering sex in local kids from the northeastern region of Mexico.

Material and methods

Animal management and study protocol was approved by the Bioethics Committee of the Facultad de Medicina Veterinaria y Zootecnia – Universidad Autónoma de Tamaulipas in the pronouncement CBBA_01_2023.

Place of study

This experiment was conducted in a commercial production unit, located in ejido Ignacio Zaragoza, Viesca, Coahuila, Mexico, within the region known as Comarca Lagunera. The climate is desert, semi-warm with cool winters (BWhw), mean annual rainfall of 240 mm, with average temperature of 25°C, ranging from -1°C in winter to 44°C in summer.

Animals and feeding

Before the experiment, during the summer, a herd of 150 local empty goats mated naturally during grazing and in housing pens.

Fourteen 57-day-old kids (7 males and 7 females) weighing 7.7 kg (live weight; LW) were housed with their mothers from birth in individual 2 x 3 m pens provided with shade, drinkers and *ad libitum* mineral salts.

Goat feeding was based on grazing from 8:00 a.m. to 1:00 p.m., and from 4:00 p.m. to 8:00 p.m., keeping the goats penned during the hottest hours of the day, and taking advantage of this space of time for kid suckling. Table 1 shows plant nutritional value during grazing.

Table 1. Average chemical composition of the main plant species consumed by local goats in northeastern Mexico.

Tabla 1. Composición química promedio de las principales especies de plantas consumidas por caprinos locales en el noreste de México.

Species	Nutritional Content (Dry basis; g kg DM)					
	DM	CP	ADF	NDF	ME	NEl
Cynodon dactilon	95.3	5.9	36.9	61.4	1.7	1.5
Amaranthus palmeri	93.8	14.4	25.9	33.7	2.1	1.3
Setaria macrostachia	94.0	13.2	37.4	61.4	1.7	1.1
Solanum eleagnifolium	93.7	22.1	32.6	37.7	2.5	1.3
Spharalcea angustifolia	94.1	11.9	39.1	51.4	2.0	1.2
Enneapogon desvauxii	94.8	5.3	44.3	71.0	1.5	0.8
Cucumis melo (Vegetative part)	90.7	13.8	33.8	34.1	2.2	1.4
Cucumis melo (Fruit)	92.9	10.5	30.9	35.7	2.2	1.1

DM= dry matter; CP= crude protein; ADF= acid detergent fiber; NDF= neutral detergent fiber; ME= metabolizable energy; NEl= net energy for lactation.

DM= materia seca; CP= proteína cruda; ADF= fibra detergente ácido; NDF= fibra detergente neutro; ME= energía metabolizable; NEl= energía neta para la lactancia.

Transportation and slaughtering of goat kids

Kids were slaughtered at weaning (57 days of age). Twelve hours before slaughter, they were separated from their mothers and transported to the Municipal slaughterhouse in Matamoros, Coahuila, where they were slaughtered following the Official Mexican Standard NOM-033-SAG/ZOO-2014.

Zoometric and morphostructural measurements

Before slaughter, zoometric traits were measured: live weight (LW), face width (FW), skull length (SL), ear length (EL), ear width (EW), neck width (NW), neck length (NL), height at withers (HW), chest circumference (CC), barrel circumference (BC), flank depth (FD), lumbosacral height (ASL), leg length (LL), cane perimeter (CP). Morphostructural traits recorded included skin pigmentation (SP), hoof pigmentation (HP), mucous membrane pigmentation (MP), presence of wattles, beard, and horns (1=present, 2=absent) (8, 12, 14).

Carcass yield

Before slaughter, the PV was recorded. Subsequently, carcass productive components were sectioned and removed (head without skin, skin, legs, lungs and trachea, liver, heart, rumen, intestine and testicles -in males-). Yield was calculated by dividing cold carcass weight (24 hours postmortem at 4°C) by the initial live weight, expressed as a percentage (4).

Nutritional value

From each kid, 200 g of *Longissimus dorsi* muscle meat were grounded to homogenize and determine protein, fat, collagen, and moisture content with a FoodScan™ Meat Analyzer.

Physicochemical characteristics of meat

To measure meat physicochemical characteristics, the *Longissimus Dorsi* muscle was removed with a transverse cut between the 12th and 14th rib, 24 hours *postmortem* (4).

pH

The pH was measured 24 h *post mortem* from a cut of the *Longissimus dorsi* muscle at the 12th rib, inserting the electrode of a portable potentiometer (HANNA® instruments, HI99163, Singapore), previously calibrated with pH 4.00 and 7.00 buffer solutions.

Color

Color was measured at three different points on the surface of the *Longissimus dorsi* muscle 24 h *post mortem*, using the Hunter method. A colorimeter (Minolta, Mod CR-400/410, Tokyo, Japan) determined L* (lightness), a* (red-green) and b* (yellow-blue) (11).

Drip loss

Drip loss was determined after Wang *et al.* (2016) with modifications. Approximately 30 g of the *Longissimus dorsi* muscle meat sample were weighed and placed in Styrofoam cups hanging from a thread without touching the walls of the cup. Subsequently, they were stored at 4°C and weighed 24 h later. Drip loss was expressed as the percentage of weight loss to initial weight (8).

Water retention capacity (WRC)

The WRC was analyzed following Guerrero *et al.* (2002), with modifications. Five g of finely minced meat of the *Longissimus dorsi* muscle, 24 h *postmortem*, were weighed and homogenized with 8 ml of sodium chloride for 1 minute using a glass rod. Subsequently, it was left to rest for 30 minutes in an ice bath. The extract was centrifuged for 25 minutes at 35,000 r.p.m. The supernatant was drained and the volume was measured in a graduated cylinder. The amount of ml of solution retained in 100 g of meat was reported.

Cooking yield

Cooking yield was determined after Liu *et al.* (2012) with modifications. From each meat sample, 50 g of the *Longissimus dorsi* muscle, 24 h *postmortem* were weighed using an analytical scale and placed in Ziploc-type bags. They were then placed in a water bath at 90°C for 15 minutes. Meat internal temperature was measured with a stem thermometer. Subsequently, they were left to rest at room temperature for 30 minutes. After this time, they were re-weighed. Cooking yield was obtained by considering initial *vs.* final weight differences, expressed as percentages.

Fatty acid (FA) profile

Fat purification was carried out through FA methylation. We proceeded to oven-dehydrate 30 g of the *Longissimus dorsi* muscle at 60°C. Subsequently, meat samples were purified (15) and methylated according to Jenkins (2010) modified by Granados-Rivera *et al.* (2017). Once the FA methyl esters were obtained, they were determined in a Hewlett Packard 6890 chromatograph with an automatic injector equipped with a silica capillary column (100 m x 0.25 mm x 0.20 μm thickness, Sp-2560, Supelco). FA identification was done by comparing retention times of each peak obtained from the chromatogram against a standard of 37 FA methyl ester components, and a specific standard for cis-9, trans-11 and trans-10, cis-12 isomers (Nu-Check).

Statistical analysis

Significant differences among quantitative zoometric traits, nutritional value, carcass and meat quality, and fatty acid profile between male and female goat kids were determined by a t-student test for independent means with the SAS version 9.3 program. Given morphostructural variables are frequencies, a Chi-square (χ^2) test was used to assess independence concerning sex.

Results

Zoometric, morphostructural, and carcass measurements

Morphostructure of local kids was heterogeneous, and no differences (P≥0.05) were found between sexes (table 2).

Table 2. Absolute (AF) and relative (RF) frequencies for morphostructural traits in local goat kids.

Tabla 2. Frecuencias absolutas (FA) y relativas (FR) para las características morfoestructurales en cabritos locales.

Skin pigmentation		**Male**	**Female**	**Mucous membrane pigmentation**		**Male**	**Female**
Present	AF (n)	3	3	Present	AF (n)	7	6
	RF (%)	21.43	21.43		RF (%)	50.0	42.86
Absent	AF (n)	4	4	Absent	AF (n)	0	1
	RF (%)	28.57	28.57		RF (%)	0	7.14
		χ^2	1.0000			χ^2	0.2994
Hoof pigmentation		**Male**	**Female**	**Presence of wattles**		**Male**	**Female**
Present	AF (n)	5	4	Present	AF (n)	1	2
	RF (%)	28.57	35.71		RF (%)	7.14	14.29
Absent	AF (n)	2	3	Absent	AF (n)	6	5
	RF (%)	21.43	14.29		RF (%)	42.86	35.71
		χ^2	0.5770			χ^2	0.5148
Presence of horns		**Male**	**Female**	**Presence of beard**		**Male**	**Female**
Present	AF (n)	4	5	Present	AF (n)	0	0
	RF (%)	28.57	35.71		RF (%)	0	0
Absent	AF (n)	3	2	Absent	AF (n)	7	7
	RF (%)	21.43	14.29		RF (%)	50.0	50.0
		χ^2	0.5770				

Concerning live weight, males were significantly heavier compared to females at 57 days old, with an average difference of 0.940 kg. Regarding other body traits, no differences (P≥0.05) were found between sexes (table 3, page 132).

Table 3. Live weight and zoometric measurements of local goat kids.
Table 3. Peso vivo y medidas zoométricas de cabritos locales.

Variable (cm)	Male	Female	SEM	*P value*
Live weight (kg)	8.23	7.29	0.3298	0.0001
Face width	6.07	5.71	0.3141	0.2842
Skull length	8.57	8.21	0.5487	0.5287
Ear length	9.42	10.07	0.1279	0.5836
Ear width	4.85	4.50	0.3891	0.3798
Neck width	17.35	17.28	1.1628	0.9522
Neck length	14.58	14.78	0.7214	0.9423
Height at withers	34.61	34.57	1.3087	0.9522
Chest circumference	32.08	30.37	0.855	0.0688
Barrel circumference	32.92	31.21	1.4487	0.2634
Flank depth	11.35	11.78	0.5933	0.4842
Lumbosacral height	35.51	34.92	1.3863	0.9600
Leg length	14.42	14.07	0.4862	0.4809
Calf perimeter	6.14	6.07	0.7641	0.7787

SEM: Mean standard error.
SEM: error estándar de la media.

Regarding yield components and carcass traits, weights of cold carcass, kidneys, head, neck, ribs, and loin were higher in males than females (P≤0.05), showing significant differences between sexes. Other carcass components did not show differences between sexes (table 4).

Table 4. Yield components of the carcass in local goat kids.
Tabla 4. Componentes de rendimiento de la canal en cabritos locales.

Variable (kg)	Male	Female	SEM	*P value*
Head	0.52	0.47	0.0192	0.0313
Skin	1.02	0.85	0.0905	0.0872
Blood	0.25	0.26	0.0497	0.7788
Hooves	0.33	0.30	0.0233	0.1674
Rumen	0.18	0.15	0.0212	0.1644
Intestine	0.27	0.23	0.0358	0.2539
Heart	0.04	0.04	0.0149	0.2577
Kidneys	0.05	0.03	0.0142	0.0285
Liver	0.16	0.14	0.0126	0.1389
Cold carcass weight	5.20	4.22	1.0222	0.0121
Omental fat	0.05	0.05	0.0136	0.6091
Mesenteric fat	0.06	0.05	0.0126	0.6095
Perinephric fat	0.11	0.07	0.0322	0.2075
Ribs	1.49	1.24	0.1025	0.0296
Legs	0.84	0.75	0.0911	0.3072
Loin	0.55	0.42	0.1013	0.0024

SEM: Mean standard error.
SEM: error estándar de la media.

Nutritional value and meat physicochemical characteristics

Meat nutritional value of male and female kids showed no differences (P≥0.05) (table 5).

Table 5. Nutritional value and meat quality of local goat kids.

Tabla 5. Valor nutricional y calidad de la carne de cabritos locales.

Variable (%)	Male	Female	SEM	*P value*
Dry matter	24.34	25.51	0.6118	0.0923
Moisture	75.65	74.48	0.6118	0.0923
Protein	21.58	22.23	0.3177	0.0648
Fat	2.49	2.18	0.2882	0.1948
Collagen	1.25	1.17	0.0983	0.4597
pH	5.99	6.15	0.2733	0.5627
Drip loss %	2.28	3.66	0.9086	0.0689
Cooking yield %	69.65	65.51	3.8076	0.2987
Water retention capacity (mL/100 g)	38.02	26.34	6.9588	0.1224
L*	48.61	48.17	1.5912	0.7835
a*	17.46	16.99	0.9604	0.6324
b*	10.06	10.18	0.8332	0.8919

L*: lightness index; a* red to green index; b*: yellow to blue index; SEM: Mean standard error.

L*: índice de luminosidad; a*índice de rojo a verde; b*: índice de amarillo a azul; SEM: error estándar de la media.

Fatty acid profile

The FAs found in greater quantity in meat were palmitic (C16:0), oleic (C18:1, n-9), stearic (C18:0), and myristic (C14:0). These, in total, represented an average of 80.85% of the FA that make up male meat and 76.83% of female meat (table 6, page 134).

Meat concentration of caproic, lauric, myristic, and oleic acids showed differences (P≥0.05) regarding sex, being higher in male meat.

The concentration of saturated FA was significantly higher in males compared to females with values of 56.45% and 46.72%, respectively, while the amount of monounsaturated (40.53 %) and polyunsaturated (9.28 %) FA was higher females compared to males (P<0.05).

Discussion

Zoometric, morphostructural and carcass measurements

Morphostructural characteristics were heterogeneous, without defined traits in terms of sex. In this regard, Maldonado-Jáquez *et al.* (2023) report that, in local kids from northern Mexico, the dominant phenotype corresponds to animals without wattles or beards. This coincides with our study since no animal presented wattles (total frequency of 78.57%), or beard. Furthermore, these same authors mention that local kids present pigmented mucous membranes and horns. In this study, 85.71% of the animals presented pigmented mucous membranes and 94.28% presented horns, reaffirming this information. These results can be attributed to local animals of this region being a cross between different breeds, with varying phenotypic traits.

On the other hand, sex had a significant effect on animal weight, where males were heavier than females. This same result is reported by Maldonado-Jáquez *et al.* (2023) for 30-day-old kids, where males weighed an average of 800 g more than females. This effect is explained by goat growth curves (2), showing shorter growth phases in males (ending up to 4 months before) than females. Moreover, the growth hormone has a marked effect on the early development of males, when the highest growth rates are observed between 20 and 60 days old (27, 29).

Regarding body measurements, our results differ from the reported by Maldonado-Jáquez *et al.* (2023), who indicated differences in neck length and width, body length, chest circumference, and leg length, probably given by age differences. While

Maldonado-Jáquez *et al.* (2023) considered 1 to 30 days, our research measured at 57 days old. Age significantly influences live weight and body conformation (3).

Cold carcass was heavier in males than in females, as found by Todaro *et al.* (2004) who reported differences in carcass weight with respect to sex with values of 5.7 kg and 5.3 in males and females of the Nebrodi breed, respectively, at 47 days old. However, Bonvillani *et al.* (2010) indicated no differences concerning sex, with average weights of 5.34 and 5.48 kg for females and males respectively in local kids from Córdoba, Argentina, at 60 to 90 days old. This could be due to age heterogeneity rather than sex. However, the breed effect could also influence carcass weight of males and females.

Differences in rib and loin weights between males and females can be attributed to males having a higher live weight at 56 days old, and consequently, a higher carcass weight reflected in a higher weight in these structures. This sex difference may assist decisions considering males being directed to the sale of cuts (30), for wholesale sale and/or in restaurants, reaching high prices. This relies on the fact that a single piece is equivalent to 50% of the price paid to the producer for a whole live goat (5). Females not meeting breeding characteristics can be commercialized as meat.

Table 6. Fatty acid profile (g/100 g^{-1} of fat) in local goat kids' meat.
Tabla 6. Perfil de ácidos grasos (g/100 g^{-1} de grasa) en carne de cabritos locales.

Fatty acid	Common name	Male	Female	SEM	*P-value*
C4:0	Butyric	0.761	1.309	0.4719	0.2675
C6:0	Caproic	0.511	0.187	0.0588	0.0005
C12:0	Lauric	1.632	0.842	0.1539	0.0003
C14:0	Myristic	9.841	6.920	0.9025	0.0071
C14:1	Myristoleic	0.339	0.511	0.0848	0.0658
C15:0	Pentadecanoic	0.826	0.570	0.1414	0.0958
C16:0	Palmitic	30.55	25.690	2.7086	0.0990
C16:1 *cis-9*	Palmitoleic	2.797	3.333	0.6165	0.4048
C17:0	Heptadecanoic	1.198	0.956	0.1728	0.1864
C17:1*cis-10*	Cis-10-Heptadecenoic	0.797	1.222	0.1971	0.0519
C18:0	Stearic	10.686	9.663	1.6219	0.5400
C18:1 *cis-9*	Elaidic	0.527	0.849	0.2435	0.2184
C18:1, n-9	Oleic	29.780	34.56	1.1955	0.0017
C18:2	Linoleic	3.697	5.353	1.1752	0.1843
C18:3 n-9,12,15	Linolenic	0.525	0.756	0.1613	0.1772
C18:2 cis-9, *trans-11*	CLA	0.283	0.305	0.0299	0.4821
C20:1 *cis-9*	Gondoic	0.048	0.044	0.0046	0.3771
C20:3 n-6	Dihomo-γ-linolenic acid	0.1130	0.149	0.0438	0.4172
C20:3 n-3	Cis 11,14,17 Eicosatrienoic	1.663	2.49	0.7037	0.2692
C22:0	Behenic acid	0.431	0.584	0.2197	0.5001
C22:6 n-3	Docosahexaenoic acid (DHA)	0.174	0.225	0.0653	0.4503
SFA		56.45	46.72	1.7464	<.0001
MUFA		34.29	40.53	1.1829	<.0001
PUFA		6.45	9.28	1.1008	<.0001
Undetermined		2.80	3.45		

[ab] Different letters in the same row show statistical differences (P ≤ 0.05); SEM: Mean standard error; SFA= saturated fatty acids, MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids.

[ab] Letras diferentes en la misma fila presentan diferencias estadísticas (P ≤ 0,05); SEM: error estándar de la media; SFA= ácidos grasos saturados, MUFA= ácidos grasos monoinsaturados; PUFA= ácidos grasos poliinsaturados.

Nutritional value of meat

Sex does not influence meat nutritional value. Other authors concluded that in Nebrodi breed kids, sex did not change the protein, fat, and ash contents of meat (34). Protein content of local kid meat from northeastern Mexico is higher than the 20.79% and 19.72%

reported by Horcada *et al.* (2012), as well as the 2.37% fat reported by Kawęcka *et al.* (2022). Fat percentage in kid meat is low compared to other species, probably because of age, since fat formed in early stages is mesenteric, while intramuscular fat is formed during adulthood (20).

Physicochemical characteristics of meat

Sex does not influence meat pH at 24 hours *post mortem*. Values found are close to those reported in Payoya kids at 30 days old (19). However, others report higher pH (23). Regardless, our values are over the recommendations for normal meat considering species for meat production (1). This effect could be due to kids being only fed with milk and muscle glycogen before slaughter is not abundant given goat restless behavior (20) rather than chronic stress before slaughter, a condition that has been documented to cause high pH values in meat (1).

Drip loss, water retention capacity, cooking yield, and color were not modified by sex, as already observed (33, 34). This may be explained by absent differences in pH since low or high final pH will determine the amount of water lost during handling, as well as pale or dark colors. The lightness index and yellow index values obtained in this study are within the reported ranks (10, 19, 34). Conversely, the values obtained for the a* parameter are higher than other reports (33, 34), probably given by factors like breed and age at the time of slaughter.

Fatty acid profile

Fatty acids found in greater quantity are within the reported ranges. Regardless of breed or feeding systems, FAs are palmitic acid (C16:0) with minimum values from 17.32 g/100 g (34) to 25.0 g/100 g (20); stearic acid (C18:0) with values from 7.87 g/100 g (21) to 19.71 g/100 g (7); and oleic acid (C18:1, n-9) with values from 25.38 g/100 g (34) to 51.08 g/100 g (21). The fact that these fatty acids predominate in meat of young and adult animals can be explained by ruminant animals with endogenous synthesis in the adipocyte from acetate, obtained during ruminal fermentation. This determines palmitic acid as the main final product, later elongated into stearic acid or desaturated to oleic acid. Long-chain fatty acids are easily synthesized in adipose tissue (17, 28).

While other studies found no differences regarding fatty acid content concerning sex in Nebrodi and Criollo Cordobes breeds (10, 34), our study found the opposite effect with a greater amount of saturated fatty acids; caproic, lauric, and myristic in male meat. While females have a higher amount of oleic unsaturated fatty acid due to the above, males showed a higher amount of saturated fatty acids and a lower amount of monounsaturated and polyunsaturated fatty acids. These differences could be due to breed or diet, since, even though kids are milk-fed, the lipid profile of the mother's milk will be largely determined by her diet (25).

Conclusions

Based on the results, we conclude that kid morphometric characteristics are heterogeneous in females and males in northeastern Mexico. Sex did not affect carcass characteristics, nutritional value, and physicochemical traits of meat. However, sex tended to modify FA profile, favoring a higher concentration of caproic (C6:0), lauric (C12:0), myristic (C14:0), and oleic (C18:1, n-9) acids in males.

This constitutes a pioneer study on morphostructure, carcass, and meat quality characterization of local goat kids from northeastern Mexico and will lay the foundations for future programs to improve the production system. The differences found could shed light on future efforts to differentiate kid meat from this region of Mexico oriented to new markets that would directly benefit small producers.

References

1. Adzitey, F.; Nurul H. 2011. Pale soft exudative (PSE) and dark firm dry (DFD) meats causes and measures to reduce these incidences-a mini review. International Food Research Journal. 18: 11-20.
2. Aguirre, L.; Albito, O.; Abad-Guamán, R.; Maza, T. 2022. Determinación de la curva de crecimiento en la cabra "Chusca Lojana" del bosque seco del Sur del Ecuador. CEDAMAZ. 12(2): 125-129. https://doi.org/10.54753/cedamaz.v12i2.1216
3. Aktas, A. H.; Gök, B.; Ates, S.; Tekin, M. E.; Halici, I.; Bas, H.; Erduran, H.; Kassam, S. 2015. Fattening performance and carcass characteristics of Turkish indigenous Hair and Honamli goat male kids. Turkish Journal of Veterinary & Animal Sciences. 39: 643-653. doi:10.3906/vet-1505-84
4. Alcalde, M. J.; Suárez, M. D.; Rodero, E.; Álvarez, R.; Sáez, M. I.; Martínez, T. F. 2017. Effects of farm management practices and transport duration on stress response and meat quality traits of suckling goat kids. Animal. 11(9): 626-1635. https://doi.org/10.1017/S1751731116002858
5. Araújo, M.; Marcílio, F.; Das- Graças, G. M.; Wandrick. H.; José, M. F.; Aldo, T. S.; Rayanna, C. F. 2017. Commercial cuts and carcass characteristics of sheep and goats supplemented with multinutritional blocks. Revista MVZ Córdoba. 22(3): 6180-6190. https://doi.org/10.21897/rmvz.1123
6. Argüello, A.; Castro, N.; Capote, J.; Solomon, M. 2005. Effects of diet and live weight at slaughter on kid meat quality. Meat Science. 70(1): 173-179. https://doi.org/10.1016/j.meatsci.2004.12.009
7. Atti, N.; Mahouachi, M.; Rouissi, H. 2006. The effect of spineless cactus (*Opuntia ficus-indica* f. inermis) supplementation on growth, carcass, meat quality and fatty acid composition of male goat kids. Meat Science. 73(2): 229-235. https://doi.org/10.1016/j.meatsci.2005.11.018
8. Bedotti, D.; Gómez-Castro, A. G.; Sánchez-Rodríguez, M.; Martos-Peinado, J. 2004. Caracterización morfológica y faneróptica de la cabra colorada pampeana. Archivos de Zootecnia. 53: 261-271. https://www.redalyc.org/pdf/495/49520303.pdf
9. BOE (Boletín Oficial del Estado). 2011. Resolución de 19 de diciembre de 2011 de la Dirección General de Recursos Agrícolas y Ganaderos, por la que se aprueba la guía del etiquetado facultativo de carne de cordero y cabrito. Núm. 314: 146362-146367. https://www.boe.es/diario_boe/txt.php?id=BOE-A-2016-12450
10. Bonvillani, A.; Peña, F.; Domenech, V.; Polvillo, O.; García, P. T.; Casal, J. J. 2010. Meat quality of Criollo Cordobes goat kids produced under extensive feeding conditions. Effects of sex and age/weight at slaughter. Spanish Journal of Agricultural Research. 8(1): 116-125. https://doi.org/10.5424/sjar/2010081-1150
11. CIE (Commission Internationale de L'Eclairage). 1986. Colorimetry (2nd ed.). Vienna.
12. Dorantes-Coronado, E. J.; Torres-Hernández, G.; Hernández-Mendo, O.; Rojo-Rubio, R. 2015. Zoometric measures and their utilization in prediction of live weight of local goats in Southern Mexico. SpringerPlus. 4: 695. https://doi.org/10. 1186/s40064-015-1424-6
13. Ekiz, B.; Ozcan, M.; Yilmaz, A.; Tölü, C.; Savaş, T. 2010. Carcass measurements and meat quality characteristics of dairy suckling kids compared to an indigenous genotype. Meat Science. 85(2): 245-249. https://doi.org/10.1016/j.meatsci.2010.01.006
14. El Moutchou, N.; González, A. M.; Chentouf, M.; Lairini, K.; Rodero, E. 2017. Morphological differentiation of Northern Morocco goat. Journal of Livestock Science and Technologies. 5(1): 33-41. https://doi.org/10.22103/JLST.2017.1662
15. Feng, S. A.; Lock, L. A.; Garnsworthy, P. C. 2004. A rapid lipid separation method for determining fatty acid composition of milk. Journal of Dairy Science. 11(87): 3785-3788. https://doi.org/10.3168/jds.S0022-0302(04)73517-1
16. Granados-Rivera, L. D.; Hernández-Mendo, O.; González-Muñoz, S. S.; Burgueño-Ferreira, J. A.; Mendoza-Martínez, G. D.; Arriaga-Jordán, C. M. 2017. Effect of palmitic acid on the mitigation of milk fat depression syndrome caused by trans-10, cis-12-conjugated linoleic acid in grazing dairy cows. Archives of animal nutrition. 71(6): 428-440. https://doi.org/10.1080/1745039X.2017.1379165
17. Granados-Rivera, L. D.; Hernández-Mendo, O. 2018. Síndrome de depresión de grasa láctea provocado por el isómero trans-10, cis-12 del ácido linoleico conjugado en vacas lactantes. Revisión. Revista mexicana de ciencias pecuarias. 9(3): 536-554. https://doi.org/10.22319/rmcp.v9i3.4337
18. Guerrero, L. I.; Ponce, A. E.; Pérez, M. L. 2002. Curso práctico de tecnología de carnes y pescado. Universidad Metropolitana Unidad Iztapalapa. D. F. México. 1: 171.
19. Guzmán, J. L.; De La Vega, F.; Zarazaga, L. Á.; Argüello, H. A.; Delgado-Pertíñez, M. 2019. Carcass characteristics and meat quality of conventionally and organically reared suckling dairy goat kids of the Payoya breed. Annals of Animal Science. 19(4): 1143-1159. https://doi.org/10.2478/aoas-2019-0047
20. Horcada, A.; Ripoll, G.; Alcalde, M. J.; Sañudo, C.; Teixeira, A.; Panea, B. 2012. Fatty acid profile of three adipose depots in seven Spanish breeds of suckling kids. Meat Science. 92(2): 89-96. https://doi.org/10.1016/j.meatsci.2012.04.018

21. Hulya, Y.; Ekiz, B.; Ozcan, M. 2018. Comparison of meat quality characteristics and fatty acid composition of finished goat kids from indigenous and dairy breeds. Tropical Animal Health Production. 50: 1261-1269. https://doi.org/10.1007/s11250-018-1553-3
22. Jenkins, T. C. 2010. Technical note: Common analytical errors yielding inaccurate results during analysis of fatty acids in feed and digesta samples. Journal of Dairy Science. 93(3):1170-1174. https://doi.org/10.3168/jds.2009-2509
23. Kawęcka, A.; Sikora, J.; Gąsior, R., Puchała, M.; Wojtycza, K. 2022. Comparison of carcass and meat quality traits of the native Polish Heath lambs and the Carpathian kids. Journal of Applied Animal Research. 50(1): 109-117. https://doi.org/10.1080/09712119.2022.2040514
24. Liu, F.; Meng, L.; Gao, X.; Li, X.; Luo, H.; Dai, R. 2012. Effect of end point temperature on cooking losses, shear force, color, protein solubility and microstructure of goat meat. Journal of Food Processing and Preservation. 37(3): 275-283. doi:10.1111/j.1745-4549.2011.00646.x
25. Madruga, M. S.; Resosemito, F. S.; Narain, N.; Souza, W. H.; Cunha, M. G. G.; Ramos, J. L. F. 2006. Effect of raising conditions of goats on physico-chemical and chemical quality of its meat. CYTAJournal of Food. 5(2): 100-104. https://doi.org/10.1080/11358120609487678
26. Maldonado-Jáquez, J. A.; Arenas-Báez, P.; Garay-Martínez, J. R.; Granados-Rivera, L. D. 2023. Body composition as a function of coat color, sex and age in local kids from northern Mexico. Agrociencia. https://doi.org/10.47163/agrociencia.v57i4.2916
27. Marquínez-Bastita, L. M.; Saldaña-Ríos, C. I.; Moreno, E. E.; Rivera, R.; Escudero, V.; Sandoya, I.; Espinosa, J.; Martínez, M. 2022. Caracterización de la producción, agroindustrialización y comercialización de ovinos y caprinos en Panamá. Ciencia Agropecuaria. 35: 30-52. http://www.revistacienciaagropecuaria.ac.pa/index.php/ciencia-agropecuaria/article/view/594
28. Martinez, A. L. M.; Alba, L. P.; Castro, G. G.; Hernández, M. P. 2010. Digestión de los lípidos en los rumiantes: una revisión. Interciencia. 35(4): 240-246. https://www.redalyc.org/pdf/339/33913156002.pdf
29. Patel, J. V.; Srivastava, A. K.; Chauhan, H. D.; Gupta, J. P.; Gami, Y. M.; Patel, V. K.; Madhavatar, M. P.; Thakkar, N. K. 2019. Factor affecting birth weight of Mehsana goat kid at organized farm. International Journal of Current Microbiology and Applied Sciences. 8(3): 1963-1967. https://doi.org/10.20546/ijcmas.2019.803.000
30. Prado, D. M.; Pozo, J. M. 2011. Acciones para potenciar el incremento de la producción y comercialización de carne ovina por el municipio de Yaguajay. Economía y Desarrollo. 146(1-2): 174-188. https://www.redalyc.org/articulo.oa?id=425541315011
31. Salinas-González, H.; Maldonado, J. A.; Torres-Hernández, G.; Triana-Gutiérrez, M.; Isidro-Requejo, L. M.; Meda-Alducin, P. 2015. Calidad composicional de la leche de cabras locales en la Comarca Lagunera de México. Revista Chapingo Serie Zonas Áridas. 14(2): 175-184. https://doi.org/10.5154/r.rchsza.2015.08.008
32. SIAP 2022. ¿Qué alimentos obtenemos de los caprinos o chivos? Consultado el 20 de agosto del 2022. https://www.gob.mx/siap/articulos/caprinos-o-chivos
33. Teixeira, A.; Jimenez-Badillo. M. R.; Rodrigues, S. 2011. Effect of sex and carcass weight on carcass traits and meat quality in goat kids of Cabrito Transmontano. Spanish Journal of Agricultural Research. (3): 753-760. https://doi.org/10.5424/sjar/20110903-248-10
34. Todaro, M.; Corrao, A.; Alicata, M. L.; Schinelli, R.; Giaccone, P.; Priolo A. 2004. Effects of litter size and sex on meat quality traits of kid meat. Small Ruminant Research. 54(3): 191-196. https://doi.org/10.1016/j.smallrumres.2003.11.011
35. Vargas-López, S.; Bustamante-González, A.; Ramírez-Bribiesca, J. E.; Torres-Hernández, G.; Larbi, A.; Maldonado-Jáquez, J. A.; López-Tecpoyotl, Z. G. 2022. Rescue and participatory conservation of Creole goats in the agro-silvopastoral systems of the Mountains of Guerrero, Mexico. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 153-162. DOI: https://doi.org/10.48162/rev.39.074
36. Wang, Z.; He, F.; Rao, W.; Ni, N.; Shen, Q.; Zhang, D. 2016. Proteomic analysis of goat Longissimus dorsi muscles with different drip loss values related to meat quality traits. Food Science and Biotechnology. 25: 425-431. https://doi.org /10.1007/s10068-016-0058

Acknowledgements

We thank the Colegio de Postgraduados- Livestock program for allowing us to carry out the fatty acid profile of the meat sample in the gas chromatograph of its Animal Nutrition laboratory.

Rev. FCA UNCuyo. 2024. 56(1): 138-148. ISSN (en línea) 1853-8665.

Effect of three different fixed-time AI protocols on follicular dynamics and pregnancy rates in suckled, dual-purpose cows in the Ecuadorian Amazon

Efecto de tres protocolos diferentes de IA a tiempo fijo sobre la dinámica folicular y las tasas de preñez en vacas de doble propósito amamantando en la Amazonía Ecuatoriana

Juan Carlos López Parra [1]†, Pablo Roberto Marini [2, 3], Alejandro Javier Macagno [4, 5*], Gabriel Amílcar Bó [4, 5]

Originales: Recepción: 13/10/2023 - Aceptación: 09/05/2024

Abstract

Reproductive performance is crucial for profitability of dual-purpose cow-calf production in the Ecuadorian Amazon. To evaluate three different fixed-time artificial insemination (FTAI) protocols in suckled, dual-purpose cows in the Ecuadorian Amazon. Lactating, Brown Swiss cows (n=301) received 2 mg estradiol benzoate (EB) and an intravaginal device containing 0.5 g of progesterone (P4) on Day 0. They were allocated randomly into the following three treatment groups: Cows in the EB group received 500 µg cloprostenol (PGF2α), and P4 devices were removed on Day 7, and 1 mg EB was administered on Day 8. Cows in the ECP group were treated as those in the EB group, except that they received 0.5 mg estradiol cypionate (ECP) at the time of P4 device removal on Day 7 instead of EB on Day 8. Cows in the J-Synch group had the P4 device removed and PGF2α administered on Day 6. All cows were FTAI on Day 9; cows in the J-Synch group also received 100 µg gonadorelin at that time. Although the diameter of the dominant follicle and the resulting CL were greater (P<0.05) in cows in the J-Synch group, pregnancies per AI did not differ (P>0.2) among groups (EB: 51.0%, ECP: 53.0% and J-Synch: 59.4%). The three protocols tested were applied successfully in suckled, dual-purpose cows with no differences in pregnancies per AI.

Keywords

prolonged proestrus • estradiol/progesterone • P4 device • FTAI • pregnancy per AI • lactating dual-purpose cows • tropical

1 Ministerio de Agricultura y Ganadería. Subsecretaría de desarrollo Pecuario. Centro Nacional de Mejoramiento Genético y Productivo El Rosario. Ecuador.
2 Universidad Nacional de Rosario. Facultad de Ciencias Veterinarias. Ovidio Lagos y Ruta 33 (2170). Argentina.
3 Universidad Nacional de Rosario. Carrera del Investigador Científico (CIC).
4 Instituto de Reproducción Animal Córdoba (IRAC). Zona Rural General Paz, C. P. 5145. Córdoba. Argentina.
5 Universidad Nacional de Villa María. Instituto A. P. de Ciencias Básicas y Aplicadas. Medicina Veterinaria. Obispo Ferreyra 411. Villa del Rosario. Córdoba. Argentina. C. P. 5963. * macagno9@gmail.com

Resumen

El desempeño reproductivo es crucial para la rentabilidad de la producción doble propósito en la Amazonía Ecuatoriana. Evaluar tres protocolos para Inseminación Artificial a tiempo fijo (IATF) en vacas en lactancia de doble propósito. Vacas Pardo Suizo en lactancia (n=301) recibieron 2 mg de benzoato de estradiol (EB) y un dispositivo intravaginal con 0,5 g de progesterona (P4) el Día 0 y fueron asignadas aleatoriamente en tres grupos. Las vacas del grupo EB recibieron 500 µg de cloprostenol (PGF2α) y se les retiró el dispositivo el Día 7, y recibieron 1 mg de EB el Día 8. Las vacas del grupo ECP fueron tratadas como las del grupo EB, excepto que recibieron 0,5 mg de cipionato de estradiol (ECP) en el día del retiro del dispositivo (Día 7) en lugar de EB el Día 8. En las vacas del grupo J-Synch, el retiro del dispositivo y la administración de PGF2α se realizaron el Día 6. Todas las vacas fueron IATF el Día 9 y las del grupo J-Synch recibieron 100 µg de gonadorelina en ese momento. Aunque el diámetro del folículo dominante y del CL fueron mayores (P<0,05) en las vacas del grupo J-Synch, las tasas de preñez a la IATF no difirieron entre los grupos (EB: 51,0%, ECP: 53,0% y J-Synch: 59,4%). Los tres protocolos probados se pueden aplicar con éxito en vacas de doble propósito amamantando.

Palabras clave

proestro prolongado • estradiol/progesterona • dispositivo de P4 • IATF • preñez por IA • vacas doble propósito en lactancia • tropical

Introduction

Dual-purpose cattle production (*i.e.*, milk and meat) represents a main source of income for small farms in tropical areas around the world, such as the Ecuadorian Amazon. However, there is a need for improvement in terms of reproductive performance and genetics in dual-purpose livestock (31). Less-than-optimal efficiency is due to multiple factors, including the environment (high temperature and humidity), nutrition, health, management, and poor animal welfare, among others (31). One of the technologies that has had the greatest impact on reproductive performance in beef and dairy cattle has been the systematic application of artificial insemination (AI), without estrus detection, known as fixed-time AI (FTAI) (4). Currently, there is a wide range of FTAI protocols used in beef and dairy cattle (4, 15, 47) which requires testing in dual-purpose cattle in the Ecuadorian Amazon. FTAI protocols are classified according to the main hormones used. Ovsynch-type protocols are based on the use of GnRH and prostaglandin F2α (PGF2α; 35, 47) and may be combined with the use of an intravaginal progesterone (P4) releasing device in beef (26, 29) and dairy cattle (41). Estradiol and P4-based protocols (2, 30) have also been commonly used for FTAI in beef and dairy cattle, especially in South America (3, 4, 48). This treatment has been simplified by the administration of estradiol cypionate (ECP) as an ovulation-inducing agent, given at the time of P4 device removal (14) replacing the use of estradiol benzoate (EB) 24 h after P4 device removal in beef (14, 44) and dairy cattle (40, 42). In general, both FTAI protocols (GnRH-based and estradiol/P4-based) result in pregnancies per AI (P/AI) between 40 to 60% in beef (1, 5) and 30 to 50% in dairy cattle (15, 43). In 2012, a new estradiol/P4-based protocol, called J-Synch, was developed (19). In this treatment protocol, the P4-releasing device is inserted for a shorter period of time (6 days) and the administration of ECP at the time of P4 device removal was replaced by the administration of GnRH at the time of AI (72 h after P4 device removal), promoting a longer proestrous period. Subsequent studies have shown that the J-Synch protocol is an efficient treatment to synchronize ovulation in beef (19, 32) and dairy (36) heifers, resulting in greater pregnancy rates than those achieved with the conventional estradiol/P4-based protocols which used ECP or EB to induce ovulation (5, 20, 36). The higher pregnancy rates obtained with the J-Synch protocol were associated with longer exposure to elevated endogenous estradiol concentrations during the proestrus period, which resulted in a more appropriate environment for early embryo development (20).

The current experiment was designed to evaluate three different estradiol/P4-based FTAI protocols, which differ mainly in the duration of the proestrus period, in suckled dual-purpose cows of the Ecuadorian Amazon. The hypothesis to be tested was that the protocol with the prolonged proestrus and utilizing GnRH to induce ovulation, named J-Synch, would result in greater P/AI than conventional estradiol/P4 protocols, which utilized EB or ECP to induce ovulation.

Materials and methods

The study was performed in the Ecuadorian Amazon, at the Center of Postgraduate Research and Conservation of the Amazon (CIPCA) of the Amazon State University in the province of Napo, which is located in the Northeast of Ecuador (Latitude: 01°14’32” South, Longitude: 53°77’13” West), from October 2015 to April 2016. The climate is tropical, with 4000 mm of precipitation per year, average relative humidity of 80% and temperatures ranging between 25 and 30°C. The altitude varies from 580 to 990 m above sea level, and although the soils have a very heterogeneous composition, most originated in fluvial sediments from the Andean plateaus.

Animals and feeding

Lactating, Brown Swiss cows (n = 301) with suckling calves were used. The study was performed in three replicates of 100 animals each. Cows were grazing a mixed pasture based on *Brachiaria decumbens* (17.585 kg DM/ha/year), *Brachiaria brizantha* (26.970 kg DM/ha/year), *Arachis pinto* (6.212 kg DM/ha/year), *Desmodium ovalifolium* (5.890 kg DM/ha/year) and *Stylosanthes guianensis* (15.237 kg DM/ha/year). They received the standard vaccination and health protocols applied commonly to cattle at the CIPCA. This included the application of vitamins and minerals, deworming, insecticide dips against ticks and flies, vaccinations for Foot and Mouth Disease, Bovine Rabies and Vesicular Stomatitis.

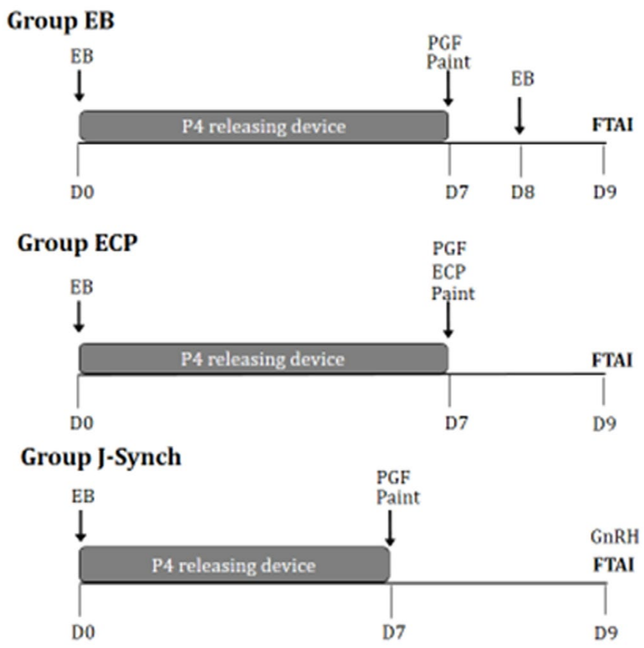
The cows were multiparous (*i.e.*, 2-5 lactations), 112.0±1.0 (mean±SEM) days postpartum, with a mean body condition score (BCS) of 2.4±0.3 (1 to 5 scale; 22), and they were producing 6.7±0.6 Kg of milk per day. Cows were selected for treatment by the presence of a CL, or at least one follicle >10 mm in diameter in their ovaries through an ultrasound examination and without any abnormalities of the reproductive tract. All cows were handled in the same milking facility and were inseminated with frozen-thawed semen from one bull of proven fertility. Animal procedures were approved and supervised by the Animal Care and Use Committee of the CIPCA and the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the University of Villa Maria (UNVM), Argentina.

Treatments

Cows were randomly allocated to one of three FTAI protocols. The EB protocol (n = 100): On Day 0, cows received a P4-releasing intravaginal device (DIB 0.5 g, Zoetis, Ecuador) plus 2 mg EB (Gonadiol, Zoetis) by intramuscular (i.m.) injection. On Day 7, the P4 device was removed, and cows received 500 μg cloprostenol (PGF2α, Ciclase DL, Zoetis) i.m. On Day 8, all cows received 1 mg EB i.m. and the tail heads were painted (Divasa - Farmavic, Spain) for the determination of estrus. On Day 9, (48 to 54 h after P4 device removal) cows were FTAI (figure 1, page 141).

The ECP protocol (n = 100): On Day 0, cows received a P4 intravaginal device plus 2 mg of EB i.m. On Day 7, the P4 device was removed, and cows received PGF2α and 0.5 mg ECP (Cipiosyn, Zoetis) i.m. and the tail heads were painted as in the EB protocol. On Day 9, (48 to 54 h after P4 device removal), cows were FTAI (figure 1, page 141).

The J-Synch protocol (n = 101): On Day 0, cows received a P4 device plus 2 mg of EB i. m. On Day 6, the P4 device was removed, cows received PGF2α i.m. and the tail heads were painted as in the previous groups. On Day 9, (72 h after P4 device removal), cows received 100 μg gonadorelin acetate (GnRH, Gonasyn gdr, Zoetis) and were FTAI concurrently (figure 1, page 141).



EB group: Day 0, P4 releasing device (0.5 g of P4) plus 2 mg EB; Day 7 device removal plus 500 µg cloprostenol (PGF); Day 8, 1 mg of EB; Day 9, FTAI (48 to 54 h after P4 device removal). ECP group: Day 0, P4 releasing device (0.5 g of P4) plus 2 mg EB; Day 7 device removal plus 500 µg cloprostenol (PGF) and 0.5 mg ECP; Day 9, FTAI (48 to 54 h after P4 device removal). J-Synch group: Day 0, P4 releasing device (0.5 g of P4) plus 2 mg EB; Day 6 device removal plus 500 µg cloprostenol (PGF); Day 9, GnRH and FTAI (72 h after device removal).

EB: Día 0, dispositivo liberador de P4 (0,5 g de P4) más 2 mg de EB; Día 7, retiro del dispositivo más 500 µg de cloprostenol (PGF); Día 8, 1 mg de EB; Día 9, IATF (48 a 54 h después de retirado el dispositivo de P4). ECP: Día 0, dispositivo liberador de P4 (0,5 g de P4) más 2 mg de EB; Día 7, retiro del dispositivo más 500 µg de cloprostenol (PGF) y 0,5 mg de ECP; Día 9, IATF (48 a 54 h después de retirado el dispositivo de P4). J-Synch: Día 0, dispositivo liberador de P4 (0,5 g de P4) más 2 mg de EB; Día 6, retiro del dispositivo más 500 µg de cloprostenol (PGF); Día 9, GnRH y IATF (72 h después de retirado el dispositivo de P4).

Figure 1. Treatment Groups.

Figura 1. Grupos de tratamiento.

Ultrasonography

All cows were examined by real-time ultrasonography (Ibex Pro and Lyte ®, USA), with a linear probe of 5.0 MHzon Days 7, 9 and 10. A map was made to measure and record all follicles >5 mm present in both ovaries, and to identify the dominant follicle which was defined as the follicle with the largest diameter at the time of device removal. To determine the timing of ovulation (disappearance of the follicle of greatest diameter) animals were examined at the time of FTAI (Day 9) and then every 12 h for the next 24 h. If the cows ovulated after Day 10, the ovulation time was defined as an ovulation occurring > 24 h after FTAI. The ovulation was reconfirmed by the presence of a CL 7 days after FTAI. Two measurements of the CL were taken using the equipment's software which included the vertical and horizontal diameter (width and height) of each CL as described previously (25).

Estrus detection

Estrus was recorded visually and by the disappearance of the tail-paint. The animals with >30% of the paint removed were considered to be in estrus (13). The visual observations 3 times a day: morning, noon and afternoon of Days 7, 8 and 9 were initiated after the removal of the P4 device.

Blood sampling and progesterone analysis

Seven days after FTAI a blood sample was taken for P4 analysis. After cleaning and disinfecting the base of the tail, samples were taken from the coccygeal vein using 10 mL Vacutainer tubes. Samples were stored at the laboratory at 4°C for 4 to 6 h after extraction and then centrifuged at 3000 RPM for 20 minutes to separate the serum. The serum was extracted and then stored at -20°C until its subsequent analysis (18). The serum P4 concentrations were determined together, in duplicates, using a progesterone ELISA kit (Progesterone Elisa, DiaMetra S.R.L., Italy). The minimum detectable P4 concentration of the kit was 0.05 ng/mL and the low and high intra-assay coefficients of variation were 4.4% and 19.5%, respectively.

Pregnancy diagnosis

The diagnosis of pregnancy was determined by ultrasonography at 35 to 40 days after FTAI.

Statistical analysis

For each continuous variable studied, the arithmetic mean (x) and the standard error (SEM) were estimated. The data were analyzed using ANOVA and means were compared by the Tukey-Kramer HSD test. Differences were considered significant with a P value of <0.05. The statistical analysis was carried out using the JMP program (JMP ®, 2003) in its version 5.0 for Windows. Estrus, ovulation and P/AI rates were compared among groups using logistic regression for binary data (*i.e.*, 1 = success, 0 = failure) and a logit link, using the Infostat program (21).

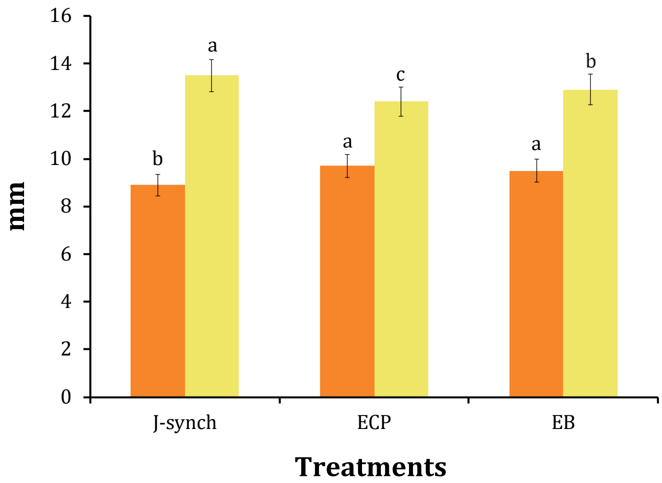
Results

Estrus expression

Overall, 90.4% (272/301) of the cows showed estrus. The percentage of cows in estrus was lower (P < 0.01) in the J-Synch treatment group (78.2%, 79/101), than in the ECP (93%, 93/100) or EB (100%, 100/100) treatment groups.

Follicular characteristics

The diameters of the dominant follicle at the time of P4 device removal and at the time of FTAI are shown in figure 2. Cows in the J-Synch group had the smallest mean diameter follicle at the time of P4 device removal and the largest diameter follicle at the time of FTAI (P < 0.05).



[abc] Different superscripts indicate significant differences between groups (P<0.05).

[abc] indica diferencias significativas entre grupos (P<0,05).

Figure 2. Diameter of dominant follicle (means ± SEM) at the time of P4 device removal (orange columns) and at the time of FTAI (yellow columns) in suckled, dual-purpose cows subjected to three different estradiol/P4-based protocols.

Figura 2. Diámetro del folículo dominante (media ± SEM) en el momento del retiro del dispositivo P4 (columnas naranjas) y en la IATF (columnas amarillas) en vacas de doble propósito sometidas a tres protocolos que utilizan estradiol/P4.

Time of ovulation

The mean interval from P4 device removal to ovulation was longer (P < 0.01) in the J-Synch group (87.7 ± 0.6 h) than in the ECP (73.7 ± 0.6 h) or EB (75.7 ± 0,6 h) groups. Overall, the percentage of cows that ovulated by 12 h after FTAI was 41.9% (126/301) and it was greater (P < 0.01) in the J-Synch group than in the other two groups (table 1). Conversely, the percentage of cows that ovulated between 12.1 and 24 h after FTAI was less in the J-Synch group than in the EB and ECP treatment groups (P < 0.01). The percentage of cows that ovulated more than 24 h after P4 device removal was 3% (9/301) and all were in the EB treatment group (9%; P > 0.1).

Table 1. Ovulatory response up to 12 h and between 12.1 and 24 h after FTAI in suckled dual-purpose cows subjected to three different estradiol/P4-based protocols.

Tabla 1. Respuesta ovulatoria hasta 12 y entre 12.1 y 24 h después de la IATF en vacas de doble propósito sometidas a diferentes protocolos que utilizan estradiol/P4.

Percentages with different superscripts differ (abP <0.01).

*Ovulations occurring within 24 h after FTAI.

Los porcentajes con diferentes superíndices indican diferencias significativas (abP <0,01).

*Ovulaciones que ocurren dentro de las 24 h posteriores a la IATF.

Treatments	n	Ovulation up to 12 h after FTAI (%)	Ovulation between 12.1 and 24 h after FTAI (%)	Acumulated ovulation* (%)
J-Synch	101	68 (67.3)^{a}	31 (30.7)^{b}	99 (98.0)
ECP	100	35 (35.0)^{b}	65 (65.0)^{a}	100 (100.0)
EB	100	23 (23.0)^{b}	68 (68.0)^{a}	91 (93.0)
Total	301	126 (41.9)	164 (54.5)	290 (97.0)

CL diameter and serum P4 concentration 7 days after FTAI

The CL diameter was greater *(P < 0.05)* in cows in the J-Synch group compared to the other two groups, which did not differ from one another (table 2). Similarly, serum P4 concentrations were higher *(P < 0.05)* in the J-Synch group than in the EB group but were intermediate in the ECP group which did not differ from either of the other treatment groups (table 2).

Table 2. CL diameter and serum progesterone concentrations 7 days after FTAI (mean ± SEM) in suckled, dual-purpose cows subjected to three different estradiol/P4-based protocols.

Tabla 2. Diámetro del CL y concentración sérica de progesterona a los siete días de la IATF (media ± SEM) en vacas de doble propósito sometidas a diferentes protocolos que utilizan estradiol/P4.

Means within columns with different superscritps differ (^{ab} P < 0.05).

Las medias con diferentes superíndices indican diferencias significativas (^{ab} P < 0,05).

Treatments	n	CL diameter (mm)	Progesterone concentration (ng/ml)
J-Synch	101	25.4 ± 0.2^{a}	11.4 ± 0.3^{a}
ECP	100	24.2 ± 0.2^{b}	10.6 ± 0.3^{ab}
EB	100	24.5 ± 0.2^{b}	9.9 ± 0.3^{b}

Pregnancies per FTAI (P/AI)

Overall, 164/301 (54.4%) of the cows were pregnant following FTAI. P/AI were numerically, but not significantly higher (P<0.2) in cows in the J-Synch group (59.4%, 60/101), compared to the EB (51.0%, 51/100) and ECP (53.0%, 53/100) treatment groups.

Another analysis investigated the effects of the diameter of the dominant follicle at the time of FTAI and the diameter of the ensuing CL on P/AI (table 3). Dominant follicle diameter at the time of FTAI was greater (P < 0.01) in cows that became pregnant than in those that did not become pregnant. Furthermore, the diameter of the CL tended (P=0.0861) to be greater in cows that became pregnant than in those that did not become pregnant to FTAI.

Table 3. Comparison of the follicular and luteal characteristics in suckled, dual-purpose cows that became pregnant or did not become pregnant following treatment with three different estradiol/P4-based FTAI protocols (Mean ± SEM).

Tabla 3. Comparación de las características foliculares y luteales entre vacas que quedaron preñadas o no, en vacas de doble propósito sometidas a tres protocolos diferentes que utilizan estradiol/P4 (media ± SEM).

Variable	Pregnancy Status*	n	Diameter (mm)	P value
DF at FTAI	P	164	13.4 ± 0.2	<0.0001
	O	137	12.3±0.3	
CL 7 days after FTAI	P	164	24.9±0.8	0.0861
	O	137	24.5±0.9	

*Pregnancy Status: (P) pregnant; (O) non-pregnant.

*Estado de gestación: (P) preñadas; (O) no preñadas.

Discussion

The environment, genetics, nutrition, health and management have been reported to be determinants of the reproductive performance in cattle. Although the prolonged proestrus protocol (J-Synch) resulted in the largest dominant follicle at the time of FTAI and the largest CL 7 days after FTAI, the hypothesis that this treatment results in greater P/AI than those in the conventional estradiol/P4 based protocols currently used in South America was not supported. Nevertheless, the results of this study have important implications because they demonstrate that other estradiol/P4-based FTAI alternatives can be applied successfully in suckled dual-purpose cattle in tropical environments. The P/AI exceeded 50% in the three treatment groups, which agrees with other studies performed on beef cattle in tropical regions (1, 5, 27, 38), but superior to those reported with dairy cattle in the same region (40, 46).

Another interesting factor related to tropical regions is the low estrus expression rate and a tendency to show estrus during the night, greatly affecting the efficiency of AI programs (4). However, in the present study, the overall estrus expression was 90.4%, which is higher than those reported for beef and dairy cattle in tropical regions (15, 33, 38, 39). Several studies have shown that the estrus expression at the time of FTAI, detected by tail-paint or estrus detection patches is associated with greater P/AI (13, 33, 38, 39, 42, 43) suggesting that tail-paint or patches are useful aids to determine that cows are indeed showing estrus at the time of FTAI, which is likely to result in improved pregnancy outcomes following FTAI.

The duration of the proestrus period tended to have an influence on P/AI in this study and has been reported to be positively related to an improved uterine environment and fertility (7, 8, 9, 11). Furthermore, the difference in dominant follicle diameter at P4 removal and at the time of FTAI in this study agrees with a similar study reported by de la Mata *et al.* (2018), where cows receiving the J-Synch treatment had the smallest follicles at P4 device removal, but had follicles of the greatest diameter at the time of FTAI compared with two other conventional (short proestrus) protocols. Although P/AI was only numerically greater in cows in the J-Synch group, the cows that became pregnant, regardless of treatment groups, had a greater follicle diameter at the time of FTAI than those cows that did not become pregnant. This is also consistent with the results reported by Yañez *et al.* (2016) on dairy cows in Ecuador. It has also been reported that *Bos taurus* beef heifers have the maximum probability of pregnancy when the follicular diameter at the time of FTAI is >12.8 mm (34). Furthermore, *Bos indicus* cows with follicles > 15 mm in diameter had the greatest probability of ovulating and becoming pregnant in estradiol/P4 based FTAI programs (38, 39). Thus, efforts to increase ovulatory follicle size would appear to be a worthy endeavor.

When the time of ovulation was evaluated in relation to the time of FTAI, the percentage of cows ovulating up to 12 h after FTAI in J-Synch treatment group was the highest (67.3%) compared to the ECP (35.0%) or EB (23.0%) treatment groups; while the percentage of cows that ovulated between 12.1 and 24 h after FTAI was 30.7% for the J-Synch treatment, and 65.0% and 68.0% for the ECP and EB treatment groups, respectively. The determination of the percentage of cows ovulating 12, 24 and after 24 h from FTAI may have important implications for fertility to FTAI programs. It has been estimated that the maximum sperm viability in the female genital tract is 24 to 30 h (23) and after ovulation occurs, the oocyte has an even shorter fertile lifespan (6). Therefore, achieving greater synchrony between ovulation and sperm arrival in the oviduct is very likely to increase P/AI (37). Although pregnancy rates did not differ among groups, inseminating cows nearer to the time of ovulation may have given an advantage to the J-Synch group; however, results did not differ significantly and increasing P/AI in the ECP and EB groups may have been due to later ovulations within 24 h after FTAI, which is still considered appropriate in cattle (16).

In this study CL size 7 days after FTAI was greater in the J-Synch treatment group than in the other groups, which is in agreement with the results reported by de la Mata *et al*. (2018). Nevertheless, it is important to note that when all cows were considered, regardless of their treatment, those that became pregnant had larger dominant follicles and tended to have larger CL than those that did not become pregnant, which is in agreement with other studies (12, 45). Thus, larger ovulatory follicles should result in larger CL and greater P/AI regardless of FTAI treatment protocol, again making this a worthy endeavor.

Endocrine and uterine environments associated with elevated estradiol concentrations before ovulation have been reported to affect the maintenance of the conceptus (9). Furthermore, preovulatory estradiol concentrations have been reported to have a positive impact on subsequent conceptus development (17). Therefore, the positive relationship between the expression of estrus, follicle diameter, plasma P4 concentration and P/AI may be explained by the effects of estradiol and P4 on the endometrial tissue, as has been shown in other studies (10, 20, 24, 28).

Conclusions

Although the prolonged proestrus protocol (J-Synch) resulted in the largest dominant follicle at the time of FTAI and the largest CL 7 days after ovulation, P/AI did not differ from the other estradiol/P4-based treatments in dual-purpose cows. Nevertheless, the present study has shown a positive association between the manifestation of estrus, follicle diameter at the time of FTAI and CL diameter 7 days later in suckled dual-purpose cows synchronized with estradiol/P4-based protocols for FTAI. Finally, it is concluded that the three protocols tested can be successfully applied in lactating dual-purpose cows in the Ecuadorian Amazon.

References

1. Baruselli, P. S.; Reis, E. L.; Marques, M. O.; Nasser, L. F.; Bó, G. A. 2004. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Animal Reproduction Science. 82-83: 479-486. DOI: 10.1016/j.anireprosci.2004.04.025
2. Bó, G. A.; Adams, G. P.; Pierson, R. A.; Mapletoft, R. 1995. Exogenous control of follicular wave emergence in cattle. Theriogenology. 43: 31-40. DOI: 10.1016/0093-691X(94)00010-R
3. Bó, G. A.; Baruselli, P. S.; Martínez, M. F. 2003. Pattern and manipulation of follicular development in Bos indicus cattle. Animal Reproduction Science. 78: 307-326. DOI: 10.1016/S0378-4320(03)00097-6
4. Bó, G. A.; Baruselli, P. S.; Mapletoft, R. J. 2013. Synchronization techniques to increase the utilization of artificial insemination in beef and dairy cattle. Animal Reproduction. 10: 137-142. DOI:10.1017/S1751731114000822

5. Bó, G. A.; de la Mata, J. J.; Baruselli, P. S.; Menchaca, A. 2016. Alternative programs for synchronizing and re-synchronizing ovulation in beef cattle. Theriogenology. 86: 388-396. DOI: 10.1016/j.theriogenology.2016.04.053
6. Brackett, B. G.; Oh, Y. K.; Evans, J. F.; Donawick, W. J. 1980. Fertilization and early development of cow ova. Biology of Reproduction. 23: 189-205. DOI: 10.1095/biolreprod23.1.189
7. Bridges, G. A.; Hesler, L. A.; Grum, D. E.; Mussard, M. L.; Gasser, C. L.; Day, M. L. 2008. Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestros increases timad-IA pregnancy rates in beef cow. Theriogenology. 69: 843-851. DOI: 10.1016/j.anireprosci.2009.05.002
8. Bridges, G. A.; Mussard, M. L.; Burke, C. R.; Day, M. L. 2010. Influence of the length of proestros on fertility and endocrine function in female cattle. Animal Reproduction Science. 117: 208-2154. DOI: 10.1016/j.anireprosci.2009.05.002
9. Bridges, G. A.; Ahola, J. K.; Brauner, C.; Cruppe, L. H.; Currin J. C.; Day, M. L.; Gunn, P. J.; Jaeger, J. R.; Lake, S. L.; Lamb, G. C.; Marquezini, G. H. L.; Peel, R. K.; Radunz, A. E.; Stevenson, J. S.; Whittier, W. D. 2012. Determination of the appropriate delivery of prostaglandin F2α in the five-day CO-Synch + controlled intravaginal drug release protocol in suckled beef cows. Journal of Animal Science. 90: 4814-4822. DOI: 10.3168/jds.2013-6845
10. Bridges, G. A.; Day, M. L.; Geary, T. W.; Cruppe, L. H. 2013a. Deficiencies in the uterine environment and failure to support embryonic development. Journal of Animal Science. 91: 3002-3013. DOI: 10.2527/jas.2013-5882
11. Bridges, G. A.; Mussard, M. L.; Helser, L. A.; Day, M. L. 2013b. Dynamics and hormone concentrations between the 7-day and 5-day CO-Synch + CIDR program in primiparous beef cows. Theriogenology. 81: 632-638. DOI: 10.1016/j.theriogenology.2013.11.020
12. Busch, D. C.; Atkins, J. A.; Bader, J. F.; Schafer, D. J.; Patterson, D. J.; Geary, T. W.; Smith, M. F. 2008. Effect of ovulatory follicle size and expression of estrus on progesterone secretion in beef cows. Journal of Animal Science. 86: 553-563. DOI: 10.2527/jas.2007-0570
13. Cedeño, A. V.; Cuervo, R.; Tríbulo, A.; Tríbulo, R.; Andrada, S.; Mapletoft, R.; Menchaca, A.; Bó, G. A. 2021. Effect of expression of estrus and treatment with GnRH on pregnancies per AI in beef cattle synchronized with an oestradiol/progesterone-based protocol. Theriogenology. 161: 294-300. DOI: 10.1016 /j.theriogenology .2020.12.014
14. Colazo, M. G.; Kastelic, J. P.; Mapletoft, R. J. 2003. Effects of oestradiol cypionate (ECP) on ovarian follicular dynamics, synchrony of ovulation, and fertility in CIDR-based, fixedtime AI programs in beef heifers. Theriogenology. 60: 855-865. DOI: 10.1016/S0093-691X(03)00091-8
15. Cosentini, C. E. C.; Wiltbank, M. C.; Sartori, R. 2021. Factors that optimize reproductive efficiency in dairy herds with emphasis on timed artificial insemination programs. Animals. 11: 301-331. DOI: 10.3390/ani11020301
16. Dalton, J. C.; Nadir, S.; Bame, J. H.; Noftsinger, M.; Nebel, R. L.; Saacke, R. G. 2001. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. J. Dairy Sci. 84: 2413-2418. DOI: 10.3168/jds.S0022-0302(01)74690-5
17. Davoodi, S.; Cooke, R. F.; Fernandes, A. C.; Cappellozza, B. I.; Vasconcelos, J. L.; Cerri, R. L. 2016. Expression of estrus modifies the gene expression profile in reproductive tissues on day 19 of gestation in beef cows. Theriogenology. 85: 645-655. DOI: 10.1016/j.theriogenology.2015.10.002
18. de Castro, T.; Valdez, L.; Rodríguez, M.; Benquet, N.; Rubianes, E. 2004. Decline in assayable progesterone in bovine serum under different storage conditions. Trop. Anim. Health Prod. 36: 381-384. DOI: 10.1023/b:trop.0000026669.06351.26
19. de la Mata, J. J.; Bó, G. A. 2012. Sincronización de celos y ovulación utilizando protocolos de benzoato de oestradiol y GnRH en periodos reducidos de inserción de un dispositivo con progesterona en vaquillonas para carn*e*. Taurus. 55: 17-23.
20. de la Mata, J. J.; Nuñez-Olivera, R.; Cuadro, F.; Bosolasco, D.; de Brun, V.; Meikle, A.; Bó, G. A.; Menchaca, A. 2018. Effects of extending the length of pro-oestrus in an ooestradioland progesterone-based oestrus synchronisation program on ovarian function, uterine environment, and pregnancy establishment in beef heifers. Reprod. Fertil. Dev. 30: 1541-1552. DOI: 10.1071/RD17473
21. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; Gonzalez, L.; Tablada, M.; Robledo, C. W. InfoStat versión 2020. Grupo InfoStat, F.C.A., Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar
22. Edmonsond, A. J.; Lean, I. J. 1989. A body condition scoring chart for Holando dairy cows. Tulare 93274 Journal of Dairy Science. 72: 68-78. DOI: 10.3168/jds.S0022-0302(89)79081-0
23. Hiers, E.; Barthle, C.; Dahms, V.; Portillo, G.; Bridges, G.; Rae, D.; Thacher, W.; Yelich, J. 2003. Synchronization of *Bos indicus x Bos taurus* cows for timed artificial insemination using gonadotropin-releasing hormone plus prostaglandina F2α in combination with melegestrol acetate. Journal Animal Science. 81: 830-835. DOI: 10.2527/2003.814830x

24. Jinks, E. M.; Smith, M. F.; Atkins, J. A.; Pohler, K. G.; Perry, G. A.; MacNeil, M. D.; Roberts, A. J.; Waterman, R. C.; Alexander, L. J.; Geary, T. W. 2013. Preovulatory oestradiol and the establishment and maintenance of pregnancy in suckled beef cows. Journal Animal Science. 91: 1176-1185. DOI: 10.2527/jas.2012-5611
25. Kastelic, J. P.; Bergefelt, D. R.; Ginther, O. J. 1990. Relationship between ultrasonic assessment of the corpus luteum and plasma progesterone concentration in heifers. Theriogenology. 33: 1269-1278. DOI: 10.1016/0093-691X(90)90045-U
26. Lamb, G. C.; Stevenson, J. S.; Kesler, D. J.; Garverick, H. A.; Brown, D. R.; Salfen, B. E. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2α for ovulation control in postpartum suckled beef cows. Journal of Animal Science. 79: 2253-2259. DOI: 10.2527/2001.7992253x
27. Madureira, G.; Consentini, C.; Motta, J.; Drum, J.; Prata, A.; Monteiro, P.; Melo, L. F.; Gonçalves, J.; Wiltbank, M.; Sartori, R. 2020. Progesterone-based timed AI protocols for Bos indicus cattle II: Reproductive outcomes of either EB or GnRH-type protocol, using or not GnRH at AI. Theriogenology. 145: 86-93. DOI: 10.1016/j.theriogenology.2020.01.033
28. Mann, G. E. 2009. Corpus luteum size and plasma progesterone concentrations in cows. Animal Reproduction Science. 115: 296-299. DOI: 10.1016/j.anireprosci.2008.11.006
29. Martínez, M. F.; Kastelic, J. P.; Adams, G. P.; Janzen, E.; McCartney, D.; Mapletoft, R. J. 2000. Estrus synchronization and fertility in beef cattle given a CIDR and oestradiol or GnRH. Can. Vet. J. 41: 786-790. PMCID: PMC1476379 PMID: 11062836
30. Martínez, M. F.; Kastelic, J. P.; Bó, G. A.; Caccia, M.; Mapletoft, R. J. 2005. Effects of ooestradiol and some of its esters on gonadotrophin release and ovarian follicular dynamics in CIDRtreated beef cattle. Anim Reprod Sci. 86: 37-52. DOI: 10.1016/j.anireprosci.2004.06.005
31. Moyano, J. C.; López, J. C.; Vargas, J.; Quinteros, O. R.; Marini, P. R. 2015. Plasmaspiegel von LH (luteinisierendes Hormon), Brunstsymptome und Qualität der Gelbkörper in verschiedenen Protokollen, zur Synchronisation der Brunst in Brown-Swiss-Milchrindern. Züchtungskunde. 87(4): 265-271.
32. Nuñez-Olivera, R.; Bó, G. A.; Menchaca, A. 2022. Association between length of proestrus, follicular size, estrus behavior, and pregnancy rate in beef heifers subjected to fixedetime artificial insemination. Association between length of proestrus, follicular size, estrus behavior, and pregnancy rate in beef heifers subjected to fixedetime artificial insemination. Theriogenology. 181: 1-7. DOI: 10.1016/j.theriogenology.2021.12.028
33. Pereira, M. H. C.; Wiltbank, M. C.; Vasconcelos, J. L. M. 2016. Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. Journal Dairy Science. 99: 237-2247. DOI: 10.3168/jds.2015-9903
34. Perry, G. A.; Smith, M. F.; Roberts, A. J.; MacNeil, M. D.; Geary, T. W. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. J. Anim. Sci. 85: 684-519. DOI: 10.2527/jas.2006-519
35. Pursley, J. R.; Mee, M. O.; Wiltbank, M. C. 1995. Synchronizatión of ovulation in dairy cows using PGF2α and GnRH. Theriogenology. 44: 915-923. DOI: 10.1016/0093-691x(95)00279-h
36. Ré, M. G.; Racca, G.; Filippi, L.; Veneranda, G.; Bó, G. A. 2021. Sincronización de la ovulación y tasas de preñez en vaquillonas lecheras tratadas con protocolos que prolongan el proestro. Taurus. 91: 28-45.
37. Saacke, R. G.; Dalton, J. C.; Nadir, S.; Nebel, R. L.; Bame, J. H. 2000. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Animal Reproduction Science. 60-61: 663-677. DOI: 10.1016/s0378-4320(00)00137-8
38. Sá Filho, M. F.; Crespilho, A. M.; Santos, J. E. P.; Perry, G. A.; Baruselli, P. S. 2010. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Animal Reproduction Science. 120: 23-30. DOI: 10.1016/j.anireprosci.2010.03.007
39. Sá Filho, M. F.; Santos, J. E. P.; Ferreira, R. M.; Sales, J. N. S.; Baruselli, P. S. 2011. Importance of estrus on pregnancy per insemination in suckled Bos indicus cows submitted to oestradiol/progesterone based timed insemination protocols. Theriogenology. 76: 455-463. DOI: 10.1016/j.theriogenology.2011.02.022
40. Souza, A. H.; Viechnieski, S.; Lima, F. A.; Silva, F. F.; Araujo, R.; Bó, G. A.; Wiltbank, M. C.; Baruselli, P. S. 2009. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. Theriogenology. 72: 10-21. DOI: 10.1016/j.theriogenology.2008.12.025
41. Stevenson, J. S.; Pursley, J. R.; Garverick, H. A.; Fricke, P. M.; Kesler, D. J.; Ottobre, J. S.; Wiltbank, M. C. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. Journal Dairy Science. 89: 2567-2578. DOI: 10.3168/jds.S0022-0302(06)72333-5
42. Tschopp, J. C.; Bó, G. A. 2022a. Success of artificial insemination based on expression of estrus and the addition of GnRH to an oestradiol/progesterone-based protocol on pregnancy rates in lactating dairy cows. Animal Reproduction Science. 238: 106954. DOI: 10.1016/j.anireprosci.2022.106954

43. Tschopp, J. C.; Macagno, A. J.; Mapletoft, R. J.; Menchaca, A.; Bó, G. A. 2022b. Effect of the addition of GnRH and a second prostaglandin F2α treatment on pregnancy per artificial insemination in lactating dairy cows submitted to an estradiol/progesterone-based timed-AI protocol. Theriogenology. 188: 63-70. DOI: 10.1016/j.theriogenology.2022.05.019
44. Uslenghi, G.; González Chavez, S.; Cabodevila, J.; Callejas, S. 2014. Effect of oestradiol cypionate and amount of progesterone in the intravaginal device on synchronization of estrus, ovulation and on pregnancy rate in beef cows treated with FTAI based protocols. Animal Reproduction Science. 145: 1-7. DOI: 10.1016/j.anireprosci.2013.12.009
45. Vasconcelos, J. L.; Sartori, R.; Oliveira, H. N.; Guenther, J. G.; Wiltbank, M. C. 2001. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pegnancy rate. Theriogenology. 56: 307-314. DOI: 10.1016/S0093-691X(01)00565-9
46. Vasconcelos, J. L. M.; Pereira, M. H. C.; Wiltbank, M. C.; Guida, T. G.; Lopes Jr, F. R.; Sanches Jr, C. P.; Pereira Barbosa, L. F.; Costa Jr, W. M.; Kloster Munhoz, A. 2018. Evolution of fixed-time AI in dairy cattle in Brazil. Animal Reproduction. 15(Suppl. 1): 940-951. DOI: 10.21451/1984-3143-AR2018-0020
47. Wiltbank, M. C.; Pursley, R. J. 2014. The cow as an induced ovulator: Timed AI after synchronization of ovulation. Theriogenology. 81: 170-185. DOI: 10.1016/j.theriogenology.2013.09.017
48. Yañez, D.; Barbona, I.; López, J. C.; Moyano, J. C.; Quinteros, R.; Bernardi, S.; Marini, P. R. 2016. Possible factors affecting pregnancy rate of cows in the amazon ecuatorian. Proceedings, VI Peruvian Congress Animal Reproduction. p. 66. DOI: 10.18548/aspe/0004.12

Author contributions

GAB: Conceptualization, Writing - review & editing; PRM: Writing - review & editing; JCLP: Supervision, Data curation, Methodology, Writing - original draft. AM: writing-review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of Funding and acknowledgments

This research was supported by Fondo Nacional de Ciencia y Tecnología (FONCYT PICT 2017-4550) of Argentina and the Center of Postgraduate Research and Conservation of the Amazon (CIPCA), Ecuador. We also thank our colleagues at the CIPCA for technical assistance and Dr. Reuben J. Mapletoft from the University of Saskatchewan for the critical review of the final version of the manuscript.

A Data Availability Statement

The data that supports this study will be shared upon reasonable request to the corresponding author.

Green solvents for the recovery of phenolic compounds from strawberry (*Fragaria* x *ananassa* Duch) and apple (*Malus domestica*) agro-industrial bio-wastes

Uso de solventes verdes para la extracción de compuestos fenólicos a partir de residuos agroindustriales de frutilla (*Fragaria* x *ananassa* Duch) y manzana (*Malus domestica*)

Esteban Villamil-Galindo [1, 2], Andrea Piagentini [1*]

Originales: *Recepción:* 25/10/2023 - *Aceptación:* 26/12/2023

Abstract

We aimed to study the obtention of valuable phenolic compounds from tissue by-products of agro-industrial processing of apple (GS) and strawberry (RF) using green solvents and Soxhlet extraction methodology. The effects of solvent type [water (W); 80% ethanol, (EtOH)] and extraction ratio (1:10, 1:20, 1:30, and 1:40 p/v) were determined on total phenolic content (TPC), antioxidant capacity (DPPH), and the profile of phenolic compounds of the GS and RF extracts. The solvent type and the extraction ratio significantly affected TPC and DPPH of GS and RF extracts. Extraction with EtOH and 1:40 ratio produced the highest yields, obtaining an RF extract with 15.8 g GAE/Kg (TPC) and 19 mmol TE/Kg (DPPH). The tetra-galloyl glucose isomer and agrimoniin (0.8-0.4 g/Kg) were the main RF phenolic compounds of the eight identified. GS extract, obtained with EtOH and 1:40 ratio, had 11.9 g GAE/Kg (TPC) and 20.5 mmol TE/Kg (DPPH), having quercetin -3-o-glucuronide (0.43 g/Kg) the highest concentration among the eight phenolic compounds identified. The results highlight the potential of green solvents to obtain valuable compounds from low-cost raw materials, like the high-antioxidant capacity phenolic compound extracts obtained herein.

Keywords
green extraction • bioactive compounds • hydrolysable tannins • flavonols • natural antioxidants

1 Universidad Nacional del Litoral. Facultad de Ingeniería Química. Instituto de Tecnología de Alimentos. Santiago del Estero 2829. 3000. Santa Fe. Argentina.
[*] ampiagen@fiq.unl.edu.ar
2 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). 3000. Santa Fe. Argentina.

Resumen

El objetivo fue estudiar la extracción de compuestos fenólicos con alto valor bioactivo, a partir de subproductos del procesamiento de manzana (GS) y frutilla (RF), utilizando solventes amigables con el medio ambiente y la extracción Soxhlet. Se determinaron los efectos del tipo de solvente [agua (W); etanol 80% (EtOH)] y la relación de extracción (1:10, 1:20, 1:30 y 1:40 p/v) sobre el contenido de fenoles totales (TPC), capacidad antioxidante (DPPH) y perfil de compuestos fenólicos. El tipo de solvente y la relación de extracción afectaron significativamente el TPC y la DPPH. La extracción con EtOH a 1:40 produjo los rendimientos más altos, obteniendo un extracto de RF con 15,8 g GAE/Kg (TPC) y 19 mmol TE/Kg (DPPH). El isómero de tetra galoil glucosa y agrimoniin (0,8-0,4 g/Kg) fueron los principales compuestos fenólicos de RF, entre los ocho identificados. El extracto de GS, obtenido con EtOH a 1:40, tuvo 11,9 g GAE/Kg (TPC) y 20,5 mmol TE/Kg (DPPH), con quercetin-3-o-glucuronido (0,43 g/Kg) como el principal compuesto fenólico entre los ocho identificados. Los resultados destacan el potencial de los solventes verdes para obtener compuestos fenólicos de alta capacidad antioxidante potencialmente bioactivos a partir de materias primas de bajo costo.

Palabras claves

extracción verde • compuestos bioactivos • taninos hidrolizables • flavonoles • antioxidantes naturales

Introduction

One-third of fruit and vegetable production is wasted or lost in the production chain, producing avoidable and non-avoidable waste (3, 31). The former includes the losses generated by wrong handling during postharvest, processing, transport, storage, and distribution, and the non-avoidable waste is that part of the vegetable or fruit that must be eliminated for processing and sale (peels, seeds, core, and inedible parts). Therefore, the appropriate disposal of this waste is essential to reduce the environmental and economic impact of agro-industrial activity. The circular economy proposes different solutions to prevent this waste plant tissue from ending up in city landfills (35). Although the composition of fruit and vegetable by-products includes vitamins, minerals, and carbohydrates (27), as well as different bioactive compounds like phenolic compounds, these residual plant tissues are mainly used as ingredients in animal feed or as a source of energy for boilers. Waste fruit and vegetable tissues are sources of phenolic compounds with significant health benefits for consumers and antioxidant properties (24, 33). Phenolic compounds are synthesised in the plant´s secondary metabolism during the normal development of plants. Their chemical structure has at least one aromatic ring with one or more hydroxyl groups, showing different biological activities according to their carbon bridges and hydroxyl substitution.

The production, consumption and industrial processing of apples and strawberries continue to increase worldwide, and so do the wasted tissues associated (12). Non-edible or non-processable fruit parts may have better composition and bioactivity than edible tissues (5, 34). The main phenolic compounds of strawberries are ellagitannins, phenolic acids and flavonoids like anthocyanins (32), and the principal apple phenolic compounds are catechins and proanthocyanidins (17), depending on the cultivar, the area, and the type of production of these crops. The extraction method of these compounds significantly impacts the extraction yields and their bioactive potential. Considering the structural diversity of the phenolic compounds, no single extraction system could produce the total recovery of these metabolites of interest from all vegetable tissues.

The solid-liquid extraction is commonly used to obtain phenolic compounds, and the success of this process depends on the plant matrix, kind of solvent, temperature, pH, and extraction technology. Polar protic solvents, like ethanol and methanol, can donate hydrogen bonds (33). Otherwise, polar aprotic solvents (acetone and ethyl acetate) have dipoles but do not possess hydrogen atoms bonded with a high electronegativity atom that can be donated in a hydrogen bond. Finally, apolar solvents (hexane and petroleum ether) have bonds between atoms with similar electronegativities (33). The extraction technique also affects phenolic compound yield. The Soxhlet extraction technique is widely used for obtaining secondary metabolites from different plant tissues, being a reference method for comparing the performance of other extraction techniques. The Soxhlet extraction has good extraction yields without using large quantities of solvent, with a total extraction time of 1-6h, including multiple extraction cycles. This technique commonly employs flammable, hazardous, and toxic organic solvents. The low production cost of ethanol (by fermentation from renewable sources), low energy requirements for final disposal and moderate chronic toxicity make it a sustainable option to replace traditional solvents, along with water, considered the greenest solvent. Water application is generally limited to low-polarity metabolites; nevertheless, it is possible to broaden the solubilisation spectrum of water with suitable parameters (8). Therefore, green extraction with safe and non-toxic solvents, like water, ethanol, and binary ethanol-water mixtures, must be studied. Both solvents are considered safe and acceptable for use in the food industry by regulatory agencies (FDA). The ethanol-aqueous solutions for polyphenol extraction from plant tissues have better yields due to their azeotropic behaviour (1). Additionally, safe solvents in Soxhlet extraction could also yield higher amounts of phenolic compounds from some agro-industrial waste (7, 8, 26).

Complementary treatments with phenolic compounds that strengthen the immune system or their use as antioxidant agents would promote obtaining these phenolic compounds of interest at a low cost, facilitating the accessibility to a larger population and valuing the wasted agro-industrial tissues using green technologies. Therefore, this work aims to study the extraction of the phenolic compounds from strawberry by-products and 'Granny Smith' apple peel, characterise the obtained extracts using the Soxhlet method, evaluate the impact of two green solvents (water and ethanol 80%), and different solid-liquid ratios on the total phenolic content, phenolic profile, and in-vitro antioxidant activity of the extracts.

Materials and methods

Plant material and Experimental design

The by-products of strawberry (RF) (*Fragaria x ananassa Duch*) cv 'Festival', consisting of sepals and stems, with non-processable parts of the fruit (part of the fruit closest to the sepal and peduncle), came from a single field (Coronda, Santa Fe, Argentina) during postharvest preparation for industrial processing. 'Granny Smith' apple peel (GS, 1 mm thickness) was obtained from the minimal processing of apples, according to Rodríguez-Arzuaga and Piagentini (2018). The RF and GS samples (89.2% and 80.4% moisture content, respectively) were weighed, packed in 40 μm polyethylene bags, and stored at -20°C until processing. Before extraction assays, both samples were ground to a particle size <1 mm.

The effect of the extraction solvent (S) and the solid-liquid ratio (R) in the phenolic compound extraction of each vegetable tissue (RF and GS) was studied through a factorial experimental design. The two experimental variables of each factorial design were S and R, with two [S: water (W) and ethanol 80% (EtOH)] and four levels [R: 1:10, 1:20, 1:30 and 1:40 w/v], respectively. The total phenolic content (TPC), phenolic compound profile, and antioxidant capacity (DPPH) were determined on each extract of RF and GS. The extraction times were four hours for EtOH extractions and eight hours for W extractions (extraction times determined in preliminary assays). The extracts were cooled (20°C), filtered, and stored at -20°C for further analysis. Each phenolic compound extraction assay was performed in triplicate.

Total phenolic content (TPC)

Each extract TPC was determined in triplicate by the Folin-Ciocalteu method (34). Gallic acid (Sigma-Aldrich, San Luis, Missouri, USA) was used as the standard reagent to perform the calibration curve, measuring the absorbance of the reaction at 760 nm in a spectrophotometer (Genesys 10s UV-Vis, Thermo Scientific™, Waltham, Massachusetts, USA). The concentration of TPC was reported as g of gallic acid equivalent (GAE) per kilogram of RF or GS (g GAE/Kg).

Phenolic compound profile

The phenolic compound profile of the extracts was performed with an LC-20AT HPLC with a photodiode array detector (PAD), with the software Lab Solutions for data processing and control (Shimadzu Co., Kyoto, Japan). The separation was performed with a hybrid reverse phase column C18 Gemini 5μ 110Å of 250×4.6 mm, attached to a guard column (Phenomenex Inc, CA, USA). The analysis was performed according to Villamil-Galindo *et al.* (2021) for RF and Villamil-Galindo and Piagentini (2022a) for GS. The quantification of the identified compounds was performed using the following external standards (Sigma-Aldrich Inc. St. Louis, Missouri, USA): Ellagic acid (EA), Kaempferol-3-O-glucoside (K3G), Quercetin-3-O-glucoside (Q3G), Chlorogenic acid (ACl), Procyanidin B2 (PACB2), (-) Epicatechin (EPQN), (+) Catechin (CQN), Floretin (FLN), Gallic acid (GA), Coumaric acid (CUA), and Ferulic acid (FRA). The results were expressed in g per Kg of tissue by-product.

Antioxidant capacity (DPPH)

The extract DPPH was determined using the 2.2-diphenyl-1-picrylhydrazyl radical (DPPH*) scavenging assay, performed by triplicate (34). A volume (200 μL) of the DPPH* methanolic solution (0.08 mM) reacts with 25 μL of extract or reference reagent 2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Sigma-Aldrich), and the absorbance was measured at 515 nm in a microplate reader (Asus UVM 340, Cambridge, England) after 2 h. The results were expressed as mmol Trolox equivalents/Kg of tissue by-product (mmol TE/Kg).

Statistical analysis

All assays were carried out in triplicates, and data were presented as the mean ± standard deviation (SD). The effect of the solid-liquid ratio and the extraction solvent on the total phenolic content, phenolic compound profile and antioxidant capacity of RS and GS extracts were determined by the analysis of variance (ANOVA). Tukey´s test (5% significance level) was used to determine the significant differences among treatment means. Besides, the correlation between the individually identified phenolic compounds and the antioxidant capacity determined in each extract was determined using Pearson's correlation test. The statistical analysis was performed with STATGRAPHICS Centurion XV software (StatPoint Technologies Inc., Warrenton, VA, USA).

Results and discussion

Phenolic compounds recovery from strawberry agro-industrial by-products (RF)

Both Soxhlet extraction parameters [solid-liquid ratio (R) and type of solvent (S)] affected ($p<0.001$) the yields of total phenolic content (TPC), the phenolic compounds profile, and the antioxidant capacity (DPPH) of the strawberry agro-industrial by-products extracts. The interaction term between R and S was also significant ($p<0.001$), meaning that R affected in a different way TPC, DPPH, and the phenolic compounds profile of the extracts depending on the type of solvent used.

The increase of R values improved the TPC yields of RF Soxhlet extraction with water (W), with the highest yield (10.69 g GAE/Kg) obtained at R 1:40, up to 48% higher than those obtained at lower R values (table 1, page 153). The concentration gradient between the solute and solvent was the driving force for the diffusion process. Therefore, better extraction yields were obtained with the highest R-value (1:40). Therefore, strawberry by-products have great potential as a source of bioactive compounds obtained with water.

The RF Soxhlet extraction with EtOH significantly increased the recovery of TPC as compared with W by 47-170% (table 1, page 153). Contrary to W extractions, R did not affect the TPC yields of the EtOH extracts (mean value 15.37 g GAE/Kg) (table 1, page 153). The phenolic compounds of RF could present preferential solvation when using water-ethanol. The compounds would show hydrophobic hydration with the few polar groups they could have in their structure, and a large amount of (-OH) groups would allow more interaction with water (6). Therefore, this phenomenon would produce more effect than the increase in the extraction ratio. However, the use of EtOH on the Soxhlet extraction system significantly increased the TPC yields compared to RF water extracts, improving extraction yields by up to 170% (table 1). Therefore, the lower the extraction ratio, the smaller the solvent needed, and the lower the production costs of the extracts.

Table 1. Total phenolic content (TPC) and antioxidant capacity (DPPH) of the extracts from strawberry by-products (RF) and 'Granny Smith' apple peel (GS).

Tabla 1. Contenido de fenoles totales (TPC) y capacidad antioxidante (DPPH) de los extractos de sub-productos de frutilla (RF) y cáscara de manzana 'Granny Smith' (GS).

Sample	R	TPC		DPPH	
		W	EtOH	W	EtOH
RF	1:10	5.58 ± 0.08^{cB}	15.10 ± 0.14^{dA}	4.82 ± 0.20^{cB}	9.95 ± 0.08^{aA}
	1:20	6.57 ± 0.23^{bcB}	15.13 ± 0.18^{dA}	10.43 ± 1.16^{bB}	15.44 ± 0.72^{bA}
	1:30	6.27 ± 0.02^{bB}	15.41 ± 0.21^{dA}	10.70 ± 0.21^{bB}	19.01 ± 0.86^{cA}
	1:40	10.69 ± 0.69^{aB}	15.82 ± 1.76^{dA}	15.34 ± 0.12^{aB}	18.99 ± 1.16^{cA}
GS	1:10	4.18 ± 0.02^{aB}	6.33 ± 0.12^{dA}	3.60 ± 0.14^{dB}	7.26 ± 0.20^{dA}
	1:20	3.90 ± 0.31^{abB}	7.27 ± 0.001^{cA}	8.22 ± 0.35^{cB}	12.89 ± 0.38^{cA}
	1:30	3.55 ± 0.13^{bB}	7.92 ± 0.09^{bA}	11.48 ± 0.39^{bB}	13.75 ± 0.15^{bA}
	1:40	4.35 ± 0.24^{aB}	11.90 ± 0.01^{aA}	14.05 ± 1.09^{aB}	20.51 ± 0.79^{aB}

Mean ± standard deviation. R: Solid-liquid ratio. W: water; EtOH: 80% ethanol. Different capital letters and lowercase letters indicate significant differences ($p<0.05$) by Tukey's test, between solvent and among different solid-liquid ratios, respectively.

Promedio ± desviación estándard. R: relación sólido-líquido. W: agua; EtOH: 80% etanol. Letras mayúscula y minúsculas indican diferencias significativas ($p<0,05$) por el test de Tukey, entre solvente y entre diferentes relaciones sólido-líquido, respectivamente.

Furthermore, these results confirm that binary ethanol-water mixtures are suitable bio-solvents for obtaining phenolic compounds due to their polar protic properties. The yields obtained for RF with EtOH using Soxhlet extraction were higher than those reported for pandan leaves (6.6 g GAE/Kg), mango by-products (4.5-6.6 g GAE/Kg), asparagus (2.8-3.7 g GAE/Kg), cauliflower (1.1-1.8 g GAE/Kg), and bergamot lemon (4-10 g GAE/Kg) (4, 14, 15, 27).

Regarding the antioxidant capacity of RF water extracts, they were significantly affected by R-value, comparable to TPC. The highest DPPH value was obtained for the 1:40 ratio (15.3 mmol TE/Kg), being up to 69% higher than that obtained at R 1:10. Otherwise, like with the content of phenolic compounds, EtOH improved the antioxidant capacity of the RF extracts obtained compared with W extracts. The EtOH RF extracts with the highest DPPH values (18.9-19.0 mmol TE/Kg) were those obtained with R 1:40 and 1:30 ($p>0.05$), respectively. The phenolic compounds are excellent antioxidants of natural origin due to their reducing- capacity, shown by the highly significant correlation between the TPC and the antioxidant capacity of the RF extracts.

Eight major phenolic compounds were identified for the strawberry agro-industrial waste tissue (RF), belonging to three main phenolic compound classes: hydrolysable tannins, ellagic acid derivatives, and flavonols (figure 1a and figure 2, page 154). Tetragalloyl-glucose isomer (TGI) and galloyl-bis-HHDP-glucose dimer (agrimoniin) (AGN) were identified among the hydrolysable tannins; ellagic acid pentoxide (EAP) and free ellagic acid (EA) among the ellagic acid derivatives; and finally, the flavonols were represented by quercetin-3-O-glucuronide (Q3G), quercetin hexoxide (QHS), kaempferol-3-O-glucuronide (K3G), and kaempferol hexoxide (KHS).

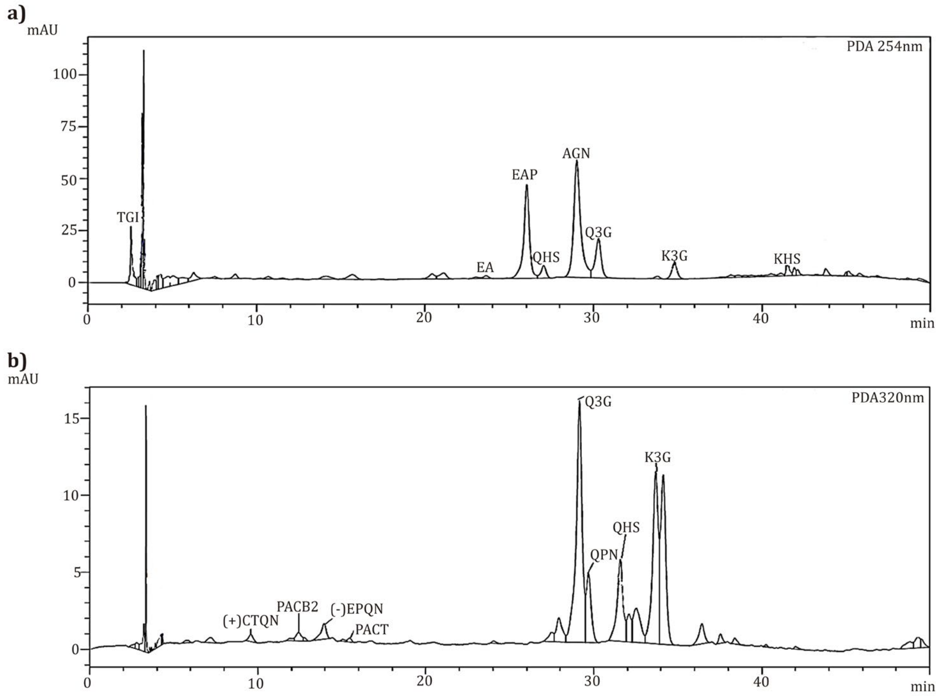


Figure 1. Typical HPLC-UV chromatogram obtained of (a) strawberry by-products at 254 nm and (b) 'Granny Smith' apple peel at 320 nm.

Figura 1. Cromatograma típico HPLC-UV obtenido para (a) sub-productos de frutillas a 254 nm y (b) cáscara de manzanas 'Granny Smith' a 320 nm.

TGI: Tetragalloyglucose isomer, **EAP**: Ellagic acid pentoxide, **AGN**: Dimer of galloyl-bis-HHDP-glucose (agrimoniin isomer), **EA**: Ellagic acid, **Q3G**: Quercetin-3-*O*-glucuronide, **QHS:** Quercetin Hexoxide, **K3G**: Kaempferol-3-*O*-glucuronide. **(+) CTQN**: Catechin, **PACB2**: Procyanidin B2, **(-) EPQN**: Epicatechin, **PACT**: Procyanidin tetramer, **QPN:** Quercetin pentoxide.

TGI: Tetragalloyglucosa isomero, **EAP**: pentoxidode ácido Ellagico, **AGN**: Dimero de galloyl-bis-HHDP-glucosa (agrimoniin isomero), **EA**: ácido Ellagico, **Q3G**: Quercetin-3-*O*-glucuronido, **QHS:** Quercetin Hexoxido, **K3G**: Kaempferol-3-*O*-glucuronido. **(+) CTQN**: Catequina, **PACB2**: Procianidina B2, **(-) EPQN**: Epicatequina, **PACT**: Procianidin tetramero, **QPN:** Quercetina pentoxido.

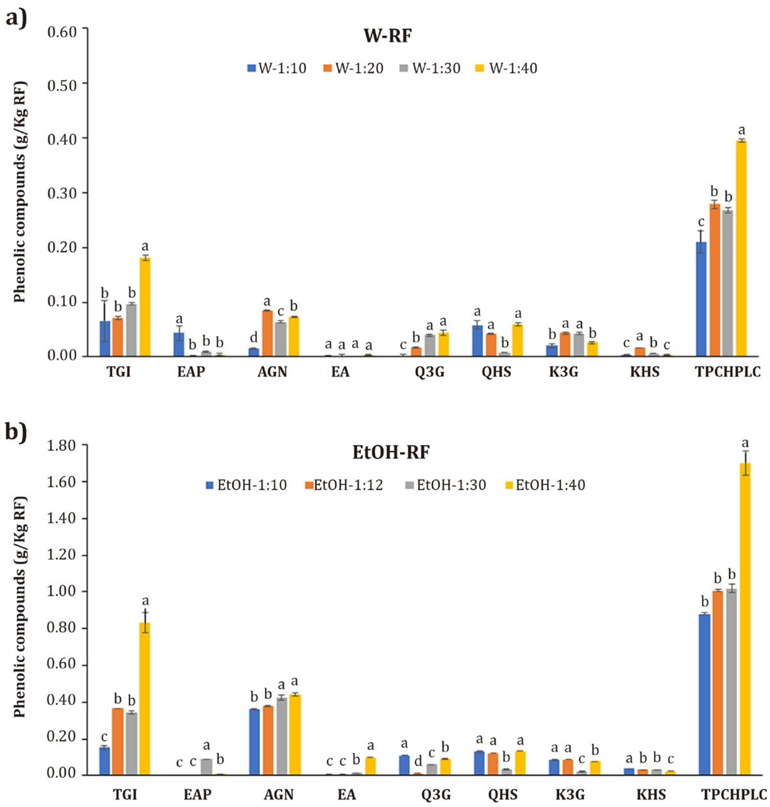


Figure 2. Phenolic compounds from strawberry by-products (RF) extracted with a) water (W) and b) 80% ethanol (EtOH).

Figura 2. Compuestos fenólicos de los sub-productos de frutilla extraídos con a) agua (W) and b) 80% etanol (EtOH).

TGI: Tetragalloyglucose isomer, **EAP**: Ellagic acid pentoxide, **AGN**: Dimer of galloyl-bis-HHDP-glucose (agrimoniin isomer), **EA**: Ellagic acid, **Q3G**: Quercetin-3-*O*-glucuronide, **QHS:** Quercetin Hexoxide, **K3G**: Kaempferol-3-*O*-glucuronide, **TPC_{HPLC}**: Total phenolic compounds analyzed by HPLC. Different lowercase letters indicate significant differences ($p<0.05$) by Tukey´s test, between different solid-liquid ratios.

TGI: Tetragalloyglucosa isomero, **EAP**: pentoxido de ácido Ellagico, **AGN**: Dimero de galloyl-bis-HHDP-glucosa (agrimoniin isomero), **EA**: ácido Ellagico, **Q3G**: Quercetin-3-*O*-glucuronido, **QHS:** Quercetin Hexoxido, **K3G**: Kaempferol-3-*O*-glucuronido, **TPC_{HPLC}**: Compuestos fenólicos totales analizados por HPLC. Diferentes letras minúsculas indican diferencias significativas ($p<0,05$) por el test de Tukey, entre diferentes relaciones sólido-líquido.

For the water extracts at R 1:10, hydrolysable tannins represented 38.5% of the total phenolic compounds, similar to flavonols with 39.9%, followed by ellagic acid derivatives with 21.6% (figure 2a, page 154). However, as the extraction ratio increased, hydrolysable tannins represented 64% of the total phenolic compounds in the extracts obtained with R 1:40, showing that the increase in the concentration gradient in the extraction solvent (W) allowed greater recovery of hydrolysable tannins (33).

The highest concentration of tetragalloylglucose isomer (TGI) in W extracts was obtained at R 1:40 (0.18 g/Kg); for the other R-values, the TGI concentration was similar (0.07-0.1 g/Kg) (figure 2a, page 154). EtOH extraction significantly improved the TGI yields for all extraction ratios, with the maximum concentration at R 1:40 (0.83 g/Kg). The Ellagic acid pentoxide (EAP) had the highest concentration, 0.088 g/Kg ($p<0.05$), in the EtOH extracts (figure 2, page 154). EAP concentrations were higher than those reported for the stem of *Sanguisorba Officinalis L* (Rosaceae family) (0.038 g/Kg) (20). Agrimoniin (AGN) is a compound derived from hexahydroxydiphenic acid (HHDP), considered a taxonomic marker of the Rosaceae, with great importance in the nutraceutical industry due to its bioactive properties (30). The AGN yield obtained with water was about 0.064-0.084 g/kg for the higher R values. The AGN yields were significantly improved for all R-values ($p<0.05$) when EtOH was used, with a maximum concentration of 0.44 g/Kg at R 1:40 (figure 2, page 154). This AGN concentration was higher than the reported for whole strawberry fruit extracts (0.12 g/Kg) obtained with 70% methanol (25), showing this residual tissue as a valuable source of AGN. The antioxidant properties of hydrolysable tannins have been reported *in-vitro* and *in-vivo* (16). Simirgiotis *et al.* (2010) reported that cyanidin glucosides and ellagic acid were the compounds with the highest participation in the antioxidant activity in the edible part of strawberries. For RF, the hydrolysable tannins TGI and AGN had a significant correlation ($p<0.01$) with the DPPH* antioxidant capacity. These compounds have polyhydric alcohol in the centre, and their hydroxyl groups could be partially esterified with ellagic acid or HHDP, having the capacity to yield electrons and thus neutralise the free radicals present (14). The ellagic acid (EA) concentrations were lower in W extracts for any extraction ratio ($p>0.05$). Like AGN, EtOH improved the extraction of EA, with a maximum concentration of 0.10 g/Kg R 1:40 (figure 2, page 154). The consumption of ellagic acid derivatives was associated with numerous health benefits since, in-vivo conditions, different types of urolithins were metabolised by the microbiota, which had powerful antiproliferative and cancer cell apoptosis-inducing activities (21).

The flavonol Quercetin-3-o-glucuronide (Q3G) concentrations in RF water extracts were 0.002-0.04 g/Kg, obtaining the higher at R 1:40 and 1:30 ($p>0.05$). Nevertheless, using 80% ethanol significantly increased Q3G yields up to 0.11 g/Kg (figure 2, page 154), similar to chokeberry extracts (11). QHS concentrations obtained with EtOH (0.12-0.13 g/Kg) were higher than those obtained with W (0.04-0.05 g/Kg). Kaempferol is one of the most common flavonols in different botanical species. In the cell vacuoles, Kaempferol tends to glycosidate with some carbohydrates to have more stability in the pH of the medium (28). The highest kaempferol-3-o-glucuronide (K3G) concentration obtained with water was 0.043 g/Kg. EtOH improved the K3G extraction yields by about 50% (figure 2, page 154). K3G values in RF were close to those reported for green tea (60% methanol), a popular antioxidant infusion (19). Finally, the Kamepferol Hexoxide (KHS) yield increased by up to 91% in EtOH extractions compared to water extracts (figure 2, page 154).

Therefore, the agro-industrial strawberry by-product showed a large variability of phenolic compounds of interest. The highest recovery of total phenolic compounds (TPC_{HPLC}) with W was achieved with R 1:40 (0.40 g/Kg). As expected, the EtOH increased TPC_{HPLC} up to 425%, obtaining the highest concentration also at R 1:40 (figure 2, page 154). Similar to the results of TPC and DPPH, the extractions with the ethanol-water binary mixture (80:20) had the highest recovery of phenolic compounds with high antioxidant capacity. The maximum TPC_{HPLC} content (1.70 g/Kg) obtained for RF EtOH extracts was comparable to that reported for strawberry plant leaves (1.95-2.07 g/Kg) obtained with methanol-formic acid (99:1) (30). The phenolic compounds of RF have an excellent antioxidant, anti-inflammatory and anticarcinogenic potential for colorectal cancer (34, 36). Therefore, the high concentration of hydrolysable tannins and ellagic acid derivatives in RF enables the promotion of this kind of agro-industrial by-products as a low-cost source of healthy compounds.

Phenolic compounds recovery from 'Granny Smith' apple peel (GS).

The solid-liquid ratio (R) and the type of solvent (S) affected ($p<0.001$) the content of phenolic compounds and the antioxidant capacity of GS extracts. The interaction term between R and S was also significant ($p<0.001$) for TPC, DPPH, and phenolic compounds profile.

The use of EtOH improved the TPC recovery from GS, like RF (table 1, page 153). The TPC of GS EtOH extracts increased as R increased, obtaining the highest yield (11.9 g GAE/Kg) for R 1:40. Castro-López *et al.* (2017) reported that R-values higher than 1:20 increased the recovery of phenolic compounds. Binary alcohol-water mixtures offer an eco-friendly solvent system for obtaining phenolic compounds from different wasted plant matrices than those using pure ethanol or other organic solvents. The water and ethanol mixture act synergistically and could provide a suitable polarity range for extracting phenolic compounds (medium-high polarity). The former is fundamental as a swelling agent of the plant matrix, allowing the lower viscosity ethanol to diffuse through the material and break the non-covalent interactions between the solute and the matrix, facilitating the preferential solvation sphere transferring the analyte to the dissolution medium (33).

Phenolic compounds are the plant-secondary metabolites with the highest reported antioxidant activity. Each compound antioxidant capacity differs due to its oxidation-reduction reactions, phenyl ring structure resonance, and hydroxyl group substitution pattern (32). The antioxidant capacity of GS extracts at R 1:40 is 47% higher in EtOH extract than in W extract. The DPPH values obtained were comparable to those reported for other plant materials by Soxhlet extraction (sugar beet molasses, rapeseed, and flowers of *Jatropha integerrima*) (10, 13). The use of EtOH enhanced the antioxidant capacity of GS extracts as R increased (table 1, page 153), comparable to TPC, and therefore, showing a strong correlation between TPC and DPPH ($p<0.01$).

The two main classes of phenolic compounds identified and quantified in 'Granny Smith' apple peel (GS) were the flavan-3-ols with (+) catechin [(+) CTQN], Procyanidin B2 (PACB2), (-) epicatechin [(-) EPQN], and Procyanidin tetramer (PACT); and the flavonols with the Quercetin-3-o-glucuronide (Q3G), Quercetin pentoxide (QPN), Quercetin Hexoxide (QHS), and Kaempferol-3-o-glucuronide (K3G) (figure 1b, page 154, and figure 3, page 157).

For the GS W extracts, the flavan-3-ols and flavanols represented each 50 % of the total phenolic compounds (R 1:10, 1:20 and 1:30), increasing the flavan-3-ols proportion with R 1:40 up to 76 % of the total of the quantified compounds, showing the affinity of this class of phenolic compounds for a polar solvent like water (figure 3a, page 157). Nevertheless, flavonols accounted for more than 50% of the total phenolic compounds in all the EtOH extractions, with a maximum of 84% in the extracts with R 1:40 (figure 3b, page 157).

R did not affect ($p>0.05$) the (+)CTQN extraction yield with water. EtOH extractions increased (+)CTQN yields up to 0.066 g/kg, 77% higher than W extracts (figure 3, page 157). The (+)CTQN yields obtained were lower than those reported by Almeida *et al.* (2017) for 'Granny Smith' apple peel extracted with 100% acetone (0.17 g/Kg). PACB2 (epicatechin-epicatechin dimer) is the most common proanthocyanidin determined in high concentrations in fruits like peaches, apples, and plums. The PACB2 content in W extracts increased with R; it was at least 71% higher for R 1:40 than for the other extraction ratios. However, ethanol did not improve the PACB2 extraction yields. Procyanidin B2 in a liquid medium from 90°C onwards starts a degradation process by oxidation and epimerisation, lowering the procyanidin B2 recovery. The highest concentration of EPQN, reported as the main phenolic compound in apples (23), was achieved with W and R 1:40 (0.1 g/Kg), being higher than that reported for apple pomace extracts (0.02 g/Kg) (20). The highest PACT concentration in W extracts was 0.04 g/Kg, obtained at R 1:40. The EtOH improved PACT yields ($p<0.05$) for all R values (figure 3, page 157). Procyanidins are oligomers composed of catechin and epicatechin; their structure and high molecular weight give them different bioactive and functional properties for the food industry.

(+)CTQN: Catechin, **PACB2**: Procyanidin B2, **(-)EPQN**: Epicatechin, **PACT**: Procyanidin tetramer, **Q3G**: Quercetin-3-*O*-glucuronide, **QPN:** Quercetin pentoxide, **QHS:** Quercetin Hexoxide, **K3G**: Kaempferol-3-*O*-glucuronide, **TPC_{HPLC}**: Total phenolic compounds analyzed by HPLC. Different lowercase letters indicate significant differences (p<0.05) by Tukey´s test, between different solid-liquid ratios.

(+)CTQN: Catequina, **PACB2**: Procianidina B2, **(-)EPQN**: Epicatequina, **PACT**: Procianidina tetramero, **Q3G**: Quercetina-3-*O*-glucuronido, **QPN:** Quercetina pentoxido, **QHS:** Quercetina Hexoxido, **K3G**: Kaempferol-3-*O*-glucuronido, **TPC_{HPLC}**: Compuestos fenólicos totales analizados por HPLC. Diferentes letras minúsculas indican diferencias significativas (p<0,05) por el test de Tukey, entre diferentes relaciones sólido-líquido.

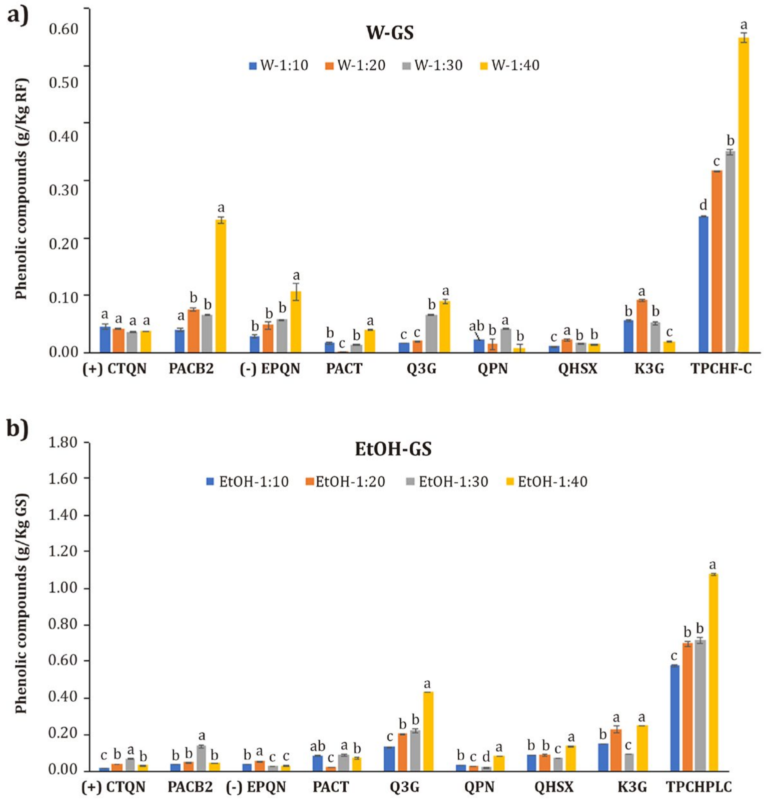


Figure 3. Phenolic compounds from 'Granny Smith' apple peel (GS) extracted with a) water (W) and b) 80% ethanol (EtOH).

Figura 3. Compuestos fenólicos de cáscara de manzana compounds from 'Granny Smith' (GS) extraidos con a) agua (W) and b) 80% etanol (EtOH).

Q3G extraction yields with W were affected by R, obtaining the highest one at R 1:40, 82% higher than the yields obtained at lower R values. Nevertheless, the EtOH improved Q3G extraction yields, as expected for a medium polarity compound. The highest Q3G yield with EtOH (0.43 g/Kg) was obtained at R 1:40, 50% higher than those obtained at lower R values (figure 3). Moreover, Q3G was the GS individual compound with the highest correlation with the antioxidant capacity, mainly for its structure and hydroxyl groups that allowed the donation of electrons, neutralising the free radicals present in the medium (32). Contrarily, RF flavonols did not show a highly significant correlation with the antioxidant capacity determined by DPPH. Consequently, the bioactive potential did not depend only on the bioactive compound concentration but also on the interaction with the food matrix. The values obtained with EtOH were similar and even higher than those reported for 'Granny Smith' apple peel acetone extracts (0.18-0.4 g/Kg) (2).

The highest concentration of QPN, the other quercetin glucoside derivative, was obtained with EtOH at R 1:40 (0.08 g/Kg). The yields of QHS in EtOH extracts were higher than in W, obtaining the highest concentration at R 1:40 (0.14 g/Kg). According to previous reports, quercetin and its glycosides have a low bioavailability (16-25%) due to their low water solubility and crystalline structure at body temperatures (16). Like the other flavonols, EtOH enhanced the recovery of K3G, with the highest concentration (0.25 g/Kg) at R 1:40 (figure 3).

Considering the sum of the compounds identified and quantified, the TPC_{HPLC} obtained for W extracts increased with R, determining the highest concentration in W at R 1:40 (0.55 g/Kg) (figure 3a). Higher TPC_{HPLC} values were obtained with EtOH and higher R values. TPC_{HPLC} extracted with EtOH 1:40 (1.07 g/Kg) was higher than that reported for the apple pulp (17), showing the bioactive potential of apple peel. The GS TPC_{HPLC} highly correlates with antioxidant activity (R^2 0.87), mainly due to flavonols. These results encourage the integral use of the apple peel as a source of valuable compounds, focusing on green solvents use with low environmental impact and cost (35).

Conclusions

There is a growing demand for nutraceutical products of vegetable origin, as their frequent consumption has been associated with a decreasing risk of having chronic non-transmissible diseases. The market for nutraceutical compounds is booming, and the extraction of bioactive compounds using clean solvents from agro-industrial waste tissues, like the strawberry by-products and apple peel, presents an opportunity to reduce costs and the environmental impact. The conventional Soxhlet extraction technique has good yields, low complexity, and high efficiency, allowing optimal use of natural resources, especially those that are rejected for industrial processing, like the waste tissue produced during the postharvest trimming of the strawberry (about 7-20% of the fruit intended for industrial processing) and Granny Smith apple peel (about 12% of the fruit intended for minimal processing).

This study demonstrates that waste vegetable tissues can be transformed into valuable phenolic compounds with antioxidant properties using eco-friendly solvents such as water and ethanol. The extracts with the highest content of phenolic compounds and antioxidant capacity were obtained for Soxhlet extraction with 80% ethanol and 1:40 extraction ratio for both the strawberry by-products (15.8 g GAE/Kg and 19 mmol TE/Kg) and the 'Granny Smith' apple peel (11.9 g GAE/Kg and 20.5 mmol TE/ Kg). Additionally, eight main phenolic compounds were identified and quantified in both waste tissues. The hydrolysable tannins, like Tetragalloyglucose isomer (TGI: 0.83 g/Kg) and Dimer of galloyl-bis-HHDP-glucose (agrimoniin isomer, AGN: 0.44 g/Kg), were the main phenolic compounds extracted from RF, while flavonols accounted for 83.7% of the total extracted phenolic compounds from GS, obtaining for Quercetin-3-O-glucuronide the highest yield (Q3G: 0.43 g/Kg).

These results demonstrated the importance of by-products as low-cost sources of bioactive compounds with high nutraceutical potential through a circular process approach in the fruit and vegetable industry. Currently, these bio-wastes are disposed of in landfills without any use. The information obtained in this study provides a pathway towards the integral use of strawberry and apple by-products. The challenge is to continue studying the development of a procedure for obtaining bioactive compounds from strawberry by-products and 'Granny Smith' apple peel with higher yields, shorter extraction times and lower energy consumption, using more sustainable and efficient technologies stimulating an integral use of these by-products.

References

1. Alara, O.; Abdurahman, N.; Ukaegbu, C. 2018. Soxhlet extraction of phenolic compounds from *Vernonia cinerea* leaves and its antioxidant activity. Journal of Applied Research on Medicinal and Aromatic Plants. 11: 12-17. https://doi.org/10.1016/j.jarmap.2018.07.003
2. Almeida, D. P. F.; Gião, M. S., Pintado, M.; Gomes, M. H. 2017. Bioactive phytochemicals in apple cultivars from the Portuguese protected geographical indication "Maçã de Alcobaça": Basis for market segmentation. International Journal of Food Properties. 20(10): 2206-2214. https://doi.org/10.1080/10942912.2016.1233431
3. Bigaran Aliotte, J. T.; Ramos de Oliveira, A. L. 2022. Multicriteria decision analysis for fruits and vegetables routes based on the food miles concept. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 54(1): 97-108. DOI: https://doi.org/10.48162/rev.39.069
4. Blancas-Benitez, F. J.; Mercado-Mercado, G.; Quirós-Sauceda, A. E.; Montalvo-González, E.; González Aguilar, G. A.; Sáyago-Ayerdi, S. G. 2015. Bioaccessibility of polyphenols associated with dietary fiber and *in vitro* kinetics release of polyphenols in Mexican "Ataulfo" mango (*Mangifera indica* L.) by-products. Food and Function. 6(3): 859-868. https://doi.org/10.1039/c4fo00982g

5. Boiteux, J.; Fernández, M. de los Á.; Espino, M.; Silva, M. F.; Pizzuolo, P. H.; Lucero, G. S. 2023. *In vitro* and in vivo efficacy of *Larrea divaricata* extract for the management of *Phytophthora palmivora* in olive trees. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 97-107. DOI: https://doi.org/10.48162/rev.39.112
6. Bosch, E.; Rosés, M. 1992. Relationship between ET polarity and composition in binary solvent mixtures. Journal of the Chemical Society, Faraday Transactions. 88(24): 3541-3546. https://doi.org/10.1039/FT9928803541
7. Bucić-Kojić, A.; Planinić, M.; Tomas, S.; Bilić, M.; Velić, D. 2007. Study of solid-liquid extraction kinetics of total polyphenols from grape seeds. Journal of Food Engineering. 81(1): 236-242. https://doi.org/10.1016/j.jfoodeng.2006.10.027
8. Carciochi, R. A.; Manrique, G. D.; Dimitrov, K. 2015. Optimization of antioxidant phenolic compounds extraction from quinoa (*Chenopodium quinoa*) seeds. Journal of Food Science and Technology. 52(7): 4396-4404. https://doi.org/10.1007/s13197-014-1514-4
9. Castro-López, C.; Ventura-Sobrevilla, J. M.; González-Hernández, M. D.; Rojas, R.; Ascacio-Valdés, J. A.; Aguilar, C. N.; Martínez-Ávila, G. C. G. 2017. Impact of extraction techniques on antioxidant capacities and phytochemical composition of polyphenol-rich extracts. Food Chemistry. 237: 1139-1148. https://doi.org/10.1016/j.foodchem.2017.06.032
10. Chen, M.; Zhao, Y.; Yu, S. 2015. Optimisation of ultrasonic-assisted extraction of phenolic compounds, antioxidants, and anthocyanins from sugar beet molasses. Food Chemistry. 172: 543-550. https://doi.org/10.1016/j.foodchem.2014.09.110
11. Ćujić, N.; Šavikin, K.; Janković, T.; Pljevljakušić, D.; Zdunić, G.; Ibrić, S. 2016. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chemistry. 194: 135-142. https://doi.org/10.1016/j.foodchem.2015.08.008
12. del Brio, D.; Tassile, V.; Bramardi, S. J.; Fernández, D. E.; Reeb, P. D. 2023. Apple (*Malus domestica*) and pear (*Pyrus communis*) yield prediction after tree image analysis. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 55(2): 1-11. DOI: https://doi.org/10.48162/rev.39.104
13. Feng, X.; Chenyi, L.; Jia, X.; Guo, Y.; Lei, N.; Hackman, R.; Chen, L.; Zhou, G. H. 2016. Influence of sodium nitrite on protein oxidation and nitrosation of sausages subjected to processing and storage. Meat Science. 116. https://doi.org/10.1016/j.meatsci.2016.01.017
14. Ghasemzadeh, A.; Jaafar, H. Z. E. 2014. Optimization of reflux conditions for total flavonoid and total phenolic extraction and enhanced antioxidant capacity in Pandan (*Pandanus amaryllifolius* Roxb.) using response surface methodology. The Scientific World Journal. 523120. https://doi.org/10.1155/2014/523120
15. Goni, I.; Hervert-Hernandez, D. 2011. By-Products from Plant Foods are Sources of Dietary Fibre and Antioxidants. Phytochemicals - Bioactivities and Impact on Health. https://doi.org/10.5772/27923
16. Grochowski, D. M.; Skalicka-Woźniak, K.; Orhan, I. E.; Xiao, J.; Locatelli, M.; Piwowarski, J. P.; Granica, S.; Tomczyk, M. 2017. A comprehensive review of agrimoniin. Annals of the New York Academy of Sciences. 1401(1): 166-180. https://doi.org/10.1111/nyas.13421
17. Kalinowska, M.; Bielawska, A.; Lewandowska-Siwkiewicz, H.; Priebe, W.; Lewandowski, W. 2014. Apples: Content of phenolic compounds *vs.* variety, part of apple and cultivation model, extraction of phenolic compounds, biological properties. Plant Physiology and Biochemistry. 84: 169e188-188. https://doi.org/10.1016/j.plaphy.2014.09.006
18. Kasikci, M. B.; Bagdatlioglu, N. 2016. Bioavailability of quercetin. Current Research in Nutrition and Food Science. 4: 146-151. https://doi.org/10.12944/CRNFSJ.4.Special-Issue-October.20
19. Kingori, S. M.; Ochanda, S. O.; Koech, R. K. 2021. Variation in Levels of Flavonols Myricetin, Quercetin and Kaempferol - In Kenyan Tea (*Camellia sinensis* L.) with Processed Tea Types and Geographic Location. Open Journal of Applied Sciences. 11: 736-749. https://doi.org/10.4236/ojapps.2021.116054
20. Lachowicz, S.; Oszmiański, J.; Rapak, A.; Ochmian, I. 2020. Profile and content of phenolic compounds in leaves, flowers, roots, and stalks of sanguisorba officinalis l. Determined with the LC-DAD-ESI- QTOF-MS/MS analysis and their *in vitro* antioxidant, antidiabetic, antiproliferative potency. Pharmaceuticals. 13(8): 1-22. https://doi.org/10.3390/ph13080191
21. Landete, J. M. 2011. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. Food Research International. 44(5): 1150-1160. https://doi.org/10.1016/j.foodres.2011.04.027
22. Li, W.; Yang, R.; Ying, D.; Yu, J.; Sanguansri, L.; Augustin, M. A. 2020. Analysis of polyphenols in apple pomace: A comparative study of different extraction and hydrolysis procedures. Industrial Crops and Products. 147. https://doi.org/10.1016/j.indcrop.2020.112250
23. Lončarić, A.; Matanović, K.; Ferrer, P.; Kovač, T.; Šarkanj, B.; Babojelić, M. S.; Lores, M. 2020. Peel of traditional apple varieties as a great source of bioactive compounds: Extraction by micro-matrix solid-phase dispersion. Foods. 9(1): 4-6. https://doi.org/10.3390/foods9010080
24. Mercado-Mercado, G.; Montalvo-González, E.; González-Aguilar, G. A.; Alvarez-Parrilla, E.; Sáyago-Ayerdi, S. G. 2018. Ultrasound-assisted extraction of carotenoids from mango (*Mangifera indica* L. 'Ataulfo') by-products on *in vitro* bioaccessibility. Food Bioscience. 21: 125-131. https://doi.org/10.1016/j.fbio.2017.12.012

25. Nowicka, A.; Kucharska, A. Z.; Sokół-Łętowska, A.; Fecka, I. 2019. Comparison of polyphenol content and antioxidant capacity of strawberry fruit from 90 cultivars of Fragaria × ananassa Duch. Food Chemistry. 270: 32-46. https://doi.org/10.1016/j.foodchem.2018.07.015
26. Oszmiański, J.; Wojdyło, A.; Gorzelany, J.; Kapusta, I. 2011. Identification and characterization of low molecular weight polyphenols in berry leaf extract by HPLC-DAD and LC-ESI/MS. Journal of Agricultural and Food Chemistry. 59: 12830-12835. https://doi.org/10.1021/jf203052j
27. Putnik, P.; Bursać Kovačević, D.; Režek Jambrak, A.; Barba, F. J.; Cravotto, G.; Binello, A.; Lorenzo, J. M.; Shpigelman, A. 2017. Innovative "green" and novel strategies for the extraction of bioactive added value compounds from citrus wastes - A review. Molecules. 22(5). https://doi.org/10.3390/molecules22050680
28. Rana, R.; Gulliya, B. 2019. Chemistry and Pharmacology of Flavonoids- A Review. Indian Journal of Pharmaceutical Education and Research. 53: 8-20. https://doi.org/10.5530/ijper.53.1.3
29. Rodríguez-Arzuaga, M.; Piagentini, A. M. 2018. New antioxidant treatment with yerba mate (*Ilex paraguariensis*) infusion for fresh-cut apples: Modeling, optimization, and acceptability. Food Science and Technology International. 24(3): 223-231. https://doi.org/10.1177/1082013217744424.
30. Simirgiotis, M. J.; Schmeda-Hirschmann, G. 2010. Determination of phenolic composition and antioxidant activity in fruits, rhizomes and leaves of the white strawberry (*Fragaria chiloensis* spp. chiloensis form chiloensis) using HPLC-DAD-ESI-MS and free radical quenching techniques. Journal of Food Composition and Analysis. 23(6): 545-553. https://doi.org/10.1016/j.jfca.2009.08.020
31. UNEP. 2021. Food Waste Index Report 2021. United Nations Environment Programme, Nairobi. https://www.unep.org/resources/report/unep-food-waste-indexreport-2021 (accessed 2 July 2022).
32. Van de Velde, F.; Grace, M. H.; Esposito, D.; Pirovani, M. É.; Lila, M. A. 2016. Quantitative comparison of phytochemical profile, antioxidant, and anti-inflammatory properties of blackberry fruits adapted to Argentina. Journal of Food Composition and Analysis. 47: 82-91. https://doi.org/10.1016/J.JFCA.2016.01.008
33. Villamil-Galindo, E.; Van de Velde, F.; Piagentini, A. M. 2020. Extracts from strawberry by-products rich in phenolic compounds reduce the activity of apple polyphenol oxidase. LWT, 133(May); 110097. https://doi.org/10.1016/j.lwt.2020.110097
34. Villamil-Galindo, E.; Van de Velde, F.; Piagentini, A. M. 2021. Strawberry agro-industrial by-products as a source of bioactive compounds: effect of cultivar on the phenolic profile and the antioxidant capacity. Bioresources and Bioprocessing. 8(1): 61. https://doi.org/10.1186/s40643-021-00416-z
35. Villamil-Galindo, E.; Piagentini, A. M. 2022a. Sequential ultrasound-assisted extraction of pectin and phenolic compounds for the valorisation of 'Granny Smith' apple peel. Food Bioscience. 49(August); 101958. https://doi.org/10.1016/j.fbio.2022.101958
36. Villamil-Galindo, E.; Antunes-Ricardo, M.; Piagentini, A. M.; Jacobo-Velázquez, D. A. 2022b. Adding value to strawberry agro-industrial by-products through ultraviolet A-induced biofortification of antioxidant and anti-inflammatory phenolic compounds. Front. Nutr. 9:1080147. doi: 10.3389/fnut.2022.1080147.

Acknowledgments

The authors acknowledge the Universidad Nacional del Litoral and the Agencia Santafesina de Ciencia, Tecnología e Innovación (ASaCTei) (Santa Fe-Argentina) for financial support through Projects CAI+D 2020 and PEICID-2022-177; the support of CONICET (Argentina) from a doctoral grant; and María del Huerto Sordo for providing strawberry by-products.