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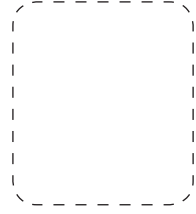
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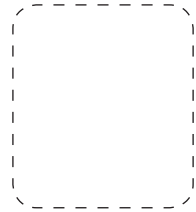


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Analysis of genetic variability *in vitro* regenerated buffelgrass plants through ISSR molecular markers

Análisis de variabilidad genética en plantas regeneradas *in vitro* de buffelgrass mediante marcadores moleculares ISSR

Edgardo Carloni ¹, Eliana López Colomba ¹, Andrea Ribotta ¹, Mariana Quiroga ¹, Exequiel Tommasino ¹, Sabrina Griffa ¹, Karina Grunberg ^{1,2}

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ABSTRACT

Genetic variability can be generated through *in vitro* culture via somaclonal variation. This tool can be potentially useful in a breeding program involving apomictic buffelgrass genotypes. The aim of this work was to evaluate inter simple sequence repeats (ISSR) as molecular markers to detect genetic variation in *in vitro* buffelgrass regenerated plants. Six plants regenerated from *in vitro* anther culture, via somatic embryogenesis were used, as well as the anther donor genotype (RN 51) as control. Of a total of 26 ISSR primers tested, 22 amplified, detecting 12% polymorphism with a divergence between 5 and 24% from RN 51. Amplification products were observed with the primers containing di-, tri- or tetra-nucleotide sequences, with or without additional nucleotides at the 3' end. The most informative primers were those containing the repetitive sequences GACA_n, AG_n or GA_n. Moreover, the regenerants transplanted at field conditions differed in morphological characteristics among them and with respect to RN 51. This study confirms that ISSR are useful to identify genetic variability in *in vitro* regenerated buffelgrass plants.

Keywords

Apomixis • somatic embryogenesis • somaclonal variation • anther culture • ISSR • genetic diversity

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RESUMEN

El cultivo *in vitro* permite generar variabilidad genética mediante variación somaclonal. Este fenómeno puede ser potencialmente útil en un programa de mejora en genotipos apomícticos de buffelgrass. El objetivo del presente estudio fue evaluar intersecuencias simples repetidas (ISSR) como marcadores moleculares en la detección de variación genética en plantas regeneradas *in vitro* de buffelgrass. Se utilizaron seis plantas regeneradas mediante el cultivo *in vitro* de anteras, vía embriogénesis somática, y el genotipo dador de anteras (NR 51) como control. De un total de 26 cebadores ISSR probados, 22 de ellos amplificaron, detectando un 12% de polimorfismo con una divergencia del 5 al 24% con respecto al material NR 51. Se observaron productos de amplificación con los cebadores que contienen secuencias di-, tri- o tetra-nucleótidos, con o sin nucleótidos adicionales en el extremo 3'. Los cebadores más informativos fueron aquellos que contenían secuencias repetitivas $GACA_n$, AG_n o GA_n . Conjuntamente, las plantas trasplantadas a campo manifestaron características morfológicas diferentes entre ellas y con respecto al NR 51. El presente estudio confirma que el empleo de ISSR permite identificar variabilidad genética en plantas regeneradas *in vitro* de buffelgrass.

Palabras claves

Apomixis • embriogénesis somática • variación somaclonal • cultivo de anteras • ISSR • diversidad genética

INTRODUCTION

In vitro cell or tissue culture offers several advantages to plant species. The use of this biotechnological tool has resulted in regenerated plants showing spontaneous genetic variability with respect to the donor plant. This phenomenon is known as somaclonal variation (20) and may be undesirable in some *in vitro* propagated species (9). In apomictic cultivars of buffelgrass [*Pennisetum ciliare* (L.) Link syn. *Cenchrus ciliaris* L.], however, those changes can be potentially useful as a source of genetic variability that can be beneficial in breeding programs (23) of this important forage grass worldwide in arid and semiarid regions (4, 18).

Genetic variation reported in several *in vitro* regenerated species include phenotypic changes (33), alterations in chromosome number or specific regions (19), point mutations (11, 24), and activation of transposable genetic elements

(21), among others. Accordingly, all these factors should be considered when identify and analyze the occurrence of a somaclonal clone (20). Morphological characters may be one of the most practical, cost-effective and easy methods to evaluate somaclonal variation (33). Nevertheless, these studies in *in vitro* regenerated plants usually require a comprehensive approach, including several nuclear DNA analysis techniques (2, 7).

In buffelgrass, measurement of nuclear DNA content by flow cytometry (FCM) has been suggested as an alternative and plays an important role in the analysis of micro-propagated plant suffering changes in ploidy levels (8). Also randomly amplified polymorphic DNA (RAPD) are mentioned to identify somaclones from induced mutations of embryogenic callus treated with ethylmethane sulfonate (EMS) and *in vitro* selection (23).

In others species, one of the approaches used in those studies is ISSR (inter simple sequence repeat) molecular markers (10, 12, 26, 30). This technique is based on polymerase chain reaction (PCR) and uses a single primer complementary to the microsatellites (28). One variant to this method consists of adding nucleotides in the 3' end of the primer, known as anchored primer (34). Each amplified band is a locus and corresponds to the DNA sequence located between two similar or inverted microsatellites. This technique has the advantage that primer design does not require previous knowledge of the DNA sequence to be amplified (5, 16). There is scarce information about the use of this type of molecular markers in buffelgrass, except for studies that determined genetic diversity in wild populations (1, 17). Here we aimed to evaluate inter simple sequence repeats (ISSR) as molecular markers to detect genetic variation in *in vitro* buffelgrass regenerated plants.

MATERIALS AND METHODS

Plant material

The study included six plants of the R₁ generation (plants obtained by *in vitro* anther culture, via somatic embryogenesis) which showed genetic instability in nuclear DNA content by flow cytometry (8) and anther donor plant registration number 51 accession (RN 51). All the plants were transplanted at field in the Instituto de Fisiología y Recursos Genéticos Vegetales of the Instituto Nacional de Tecnología Agropecuaria (INTA) (Córdoba, Argentina) for further evaluation.

Inter simple sequence repeats (ISSR)

Genomic DNA of each regenerated and donor plant was isolated from frozen leaves (-20°C) using the *Nucleon PhytoPure*

(GE Healthcare) commercial kit and following the manufacturer's instructions. Pure DNA was resuspended in 50 µl of distilled water. DNA concentration in each sample was quantified by absorbance reading (A_{260/280} nm) in a *NanoDrop* ND-1000 V3.5 spectrophotometer (NanoDrop Technologies, USA).

A total of 26 ISSR universal primers were tested; they were obtained from sequences published on line the "UBC Primer Set #9" (31) and from previous works in other plant species (14, 26, 32) (table 1, page 4).

The amplification reaction was performed in a final volume of 20 µl, containing 2 µl DNA (25 ng/µl), 1X buffer solution (Tris-HCl 6 mM pH 8.8, KCl 50 mM), 0.8 µl MgCl₂ (25 mM), 4 µl of each dNTP (1 mM), 4 µl of the primer (5 µM) and 0.2 U of GoTaq[®] DNA polymerase (Promega, USA). Amplification consisted of following steps: 94°C for 3 min, 35 cycles at 93°C for 30 s, 55°C for 1 min, 72°C for 1 min 30 s, followed by a final extension of 72°C for 5 min. To improve the band patterns of those primers that failed to amplify, different annealing temperatures were tested (14) and in some reactions, 0.5 µl (10 mg/ml) of bovine serum albumin (BSA) was added. A minimum of three reactions per primer were performed to increase resolution of the band pattern. Amplifications were conducted in an Eppendorf Mastercycler 5333 thermocycler.

Amplification products were run for two hours at 100 V in 2% agarose gels in 0.5 X TBE buffer solution. A sample volume of 12 µl was loaded per lane [10 µl amplification product + 2 µl 5X loading buffer] as well as a GeneRuler 100-pb molecular weight marker, *Plus DNA Ladder* (Thermo Scientific Inc) as reference marker to calculate the size of the bands obtained with the ISSR.

Table 1. Analysis of genetic variability of *in vitro* regenerated plants buffelgrass by ISSR.**Tabla 1.** Análisis de la variabilidad genética en plantas regeneradas *in vitro* de buffelgrass mediante ISSR.

N°	Primers	Nuc. Seq.	Temp.	PB	MB	TB	PMF (95)	PIC	AMP	SB
1	17899	(CA) ₆ AG	55°C + BSA	2	19	21	0.10	0.21	95	350≤3000
2	AE ₂	(CA) ₈ G	55°C	1	25	26	0.04	0.21	99	100≤1400
3	D12	(GA) ₆ CG	55°C + BSA	0	20	20	0.00	nd	100	200≤2900
4	D14	(CAC) ₃ GC	52°C and 55°C	-	-	-	-	-	-	-
5	HB13	(GAG) ₃ GC	52°C + BSA	0	25	25	0.00	nd	100	200≤3000
6	HB14	(CTC) ₃ GC	55°C + BSA	0	3	3	0.00	nd	100	400≤700
7	ISSR1A	(AG) ₈ Y*	55°C	11	18	29	0.38	0.23	84	100≤1800
8	ISSR1B	(AG) ₈ GG	52°C + BSA	1	9	10	0.10	0.21	99	180≤550
9	ISSR4	(GA) ₈ T	55°C + BSA	6	9	15	0.40	0.21	85	220≤1500
10	ISSR8A	(AG) ₈ Y*T	55°C + BSA	5	23	28	0.18	0.24	92	200≤3000
11	ISSR9A	(GATC) ₃ GC	55°C + BSA	0	21	21	0.00	nd	100	250≤3000
12	ISSR9B	(AG) ₈ Y*C	55°C + BSA	1	24	25	0.04	0.21	97	250≤1800
13	RAF16	(GACA) ₄	52°C + BSA	1	25	26	0.04	0.32	99	250≤3000
14	RAF4	(GA) ₉ T	55°C	4	11	15	0.27	0.21	91	400≤2300
15	RAF9	(CAA) ₅	55°C + BSA	0	11	11	0.00	nd	100	280≤3000
16	UBC816	(CA) ₈ T	55°C + BSA	3	25	28	0.11	0.21	96	250≤2500
17	UBC825	(AC) ₈ T	55°C + BSA	0	21	21	0.00	nd	100	180≤2000
18	UBC827	(AC) ₈ G	55°C	5	21	26	0.19	0.21	92	250≤3000
19	UBC834	(AG) ₆ CT	55°C + BSA	2	19	21	0.10	0.21	95	200≤1800
20	UBC840A	(GA) ₈ CT	55°C	11	21	32	0.34	0.24	89	180≤3000
21	UBC840B	(GA) ₈ TT	52°C + BSA	4	22	26	0.15	0.21	92	200≤2000
22	UBC850	(GT) ₈ Y*C	55°C	0	17	17	0.00	nd	100	300≤1500
23	UBC861	(ACC) ₆	52°C and 55°C	-	-	-	-	-	-	-
24	UBC863	(AGT) ₆	52°C and 55°C	-	-	-	-	-	-	-
25	UBC864	(ATG) ₆	55°C + BSA	1	13	14	0.07	0.21	99	180≤2000
26	UBC865	(CCG) ₆	52°C and 55°C	-	-	-	-	-	-	-

Primers, nucleotide sequences (Nuc. Seq.), annealing temperatures (Temp.), number of polymorphic bands (PB), number of monomorphic bands (MB), number of total bands (TB), proportion of polymorphic loci (PMF(95)), polymorphic information content (PIC), amplification percentage (AMP) and size of bands (SB) generated by the ISSR primers. (*) Y= T/C. (-) Failed to amplify. nd: no data available.

Cebadores, secuencias de nucleótidos (Nuc. Seq.) temperaturas de hibridación (Temp.), número de bandas polimórficas (PB), número de bandas monomórficas (MB), número de bandas totales (TB), proporción de bandas polimórficas (PMF(95)), contenido de información polimórfica (PIC), porcentaje de amplificación (AMP) y tamaño de bandas (SB) generadas por los cebadores ISSR. Y= T/C. (-) No amplificó. nd: datos no disponibles.

PCR products were stained with ethidium bromide (10 mg/ml, GIBCO BRL) and fragments were observed under UV light (312 nm). Finally, the gel images were captured using BIORAD, Molecular Imager® Gel Doc™ XR System (USA).

Phenotypic evaluation

To identify the occurrence and observe phenotypic changes, RN 51 used as control and *in vitro* regenerated plants were transferred to an experimental plot in a completely randomized design. Thus, plant materials were transplanted at 1 m distance between them and morphological characters of leaf, stem and panicle were measured (25).

Phenotypic tests were performed during two crop seasons (2013/14 and 2014/15).

Statistical Analyses

The molecular profiles obtained using ISSR were coded by performing a matrix of binary data per primer and genotypes, where "1" and "0" indicated presence and absence of bands, respectively, and each band was regarded as a locus. Genetic relationships between individuals were evaluated using the Jaccard's similarity index [$J = a / (a + b + c)$] (15, 26, 27), where a, b and c are absolute frequencies for events (1.1), (1.0) and (0.1), respectively. To obtain a matrix of genetic distances between individuals a transformation of the Jaccard's index (sqrt (1-S)) was performed. From the obtained data the proportion of polymorphic loci (PMF (95)) and polymorphism information content (PIC) were calculated. The PMF was calculated as the number of polymorphic loci / total number of loci, while PIC was estimated using the following equation:

$$PIC = 1 - \left(\sum_{i=1}^n p_i^2 \right) - \sum_{j=i+1}^n \sum_{j=i+1}^n 2p_i^2 p_j^2$$

where:

p_i = the frequency of the

i = allele

n = the number of alleles (6)

The distance matrix was subjected to a principal coordinate analysis (PCoA) and to unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering. All the analyses conducted with the binary data matrix were performed using Info-Gen statistical software (3).

To confirm phenotypic variability in the *in vitro* regenerated materials, morphological variables of R_1 plants were compared with those of the anther-donor plant (RN 51) using *t* test. To determine the degree of grouping of R_1 and RN 51 material, a clustering analysis was performed using the average linking clustering UPGMA by applying the Euclidean distance as proximity measure. The analyses were performed using InfoStat statistical software (13).

RESULTS

Inter simple sequence repeats (ISSR)

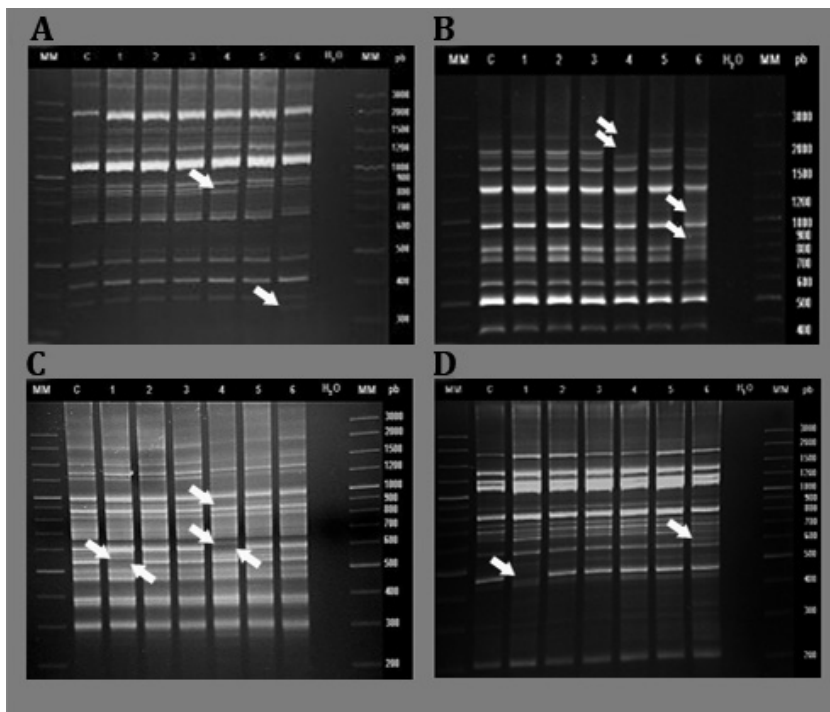
The ISSR generated amplification products in the DNA samples extracted from buffelgrass plants. Of a total of 26 primers tested, 22 amplified clear and reproducible bands. Table 1 (page 4) shows a descriptive analysis of amplification data obtained with ISSR primers. A total of 460 bands were generated, of which 58 were polymorphic (12%). Fifteen (68%) polymorphic primers were detected, with a proportion from 4 to 40% of polymorphic bands. The primers RAF16 (0.32), ISSR8A (0.24), UBC840A (0.24) and ISSR1A (0.23) produced the highest polymorphism information content. Of them, ISSR1A and UBC840A had the highest proportion of polymorphic bands (0.38 and 0.34, respectively).

Most of the polymorphic ISSR (RAF4, UBC834, ISSR8A, 17899, UBC827, UBC840A, ISSR1A, UBC816, ISSR4) generated the two types of expected data (absence and presence of alleles).

However, in comparison to donor plant, some primers only showed absence of alleles (AE₂, UBC864, ISSR1B and RAF16), whereas ISSR9B showed presence of new alleles.

The amplification profiles generated with ISSRs 17899, RAF4, UBC827 and UBC834 are displayed as an example in figure 1.

To understand the degree of similarity of molecular profiles between the *in vitro* regenerated individuals (R₁) and RN 51, the 22 ISSR that amplified were used for diversity studies and distance analyses.



MM: Molecular weight marker (100 pb Plus DNA Ladder, Thermo Scientific). C: DNA of anther donor plant (RN 51) used as control. Lanes 1 to 6: DNA of 6 *in vitro* regenerated buffelgrass plants, corresponding to the materials R_{1-14'}, R_{1-15'}, R_{1-16'}, R_{1-131'}, R₁₋₁₃₂ y R_{1-133'}, respectively. H₂O: Negative control (a reaction containing all PCR components except the DNA template of the studied plant). The arrows indicate absence or presence of bands.

MM: Marcador de peso molecular (100 pb Plus DNA Ladder, Thermo Scientific). C: ADN de planta dadora de anteras (NR 51) utilizada como control. Calles 1 al 6: ADN de 6 plantas regeneradas *in vitro* de buffelgrass, correspondiente a los materiales R_{1-14'}, R_{1-15'}, R_{1-16'}, R_{1-131'}, R₁₋₁₃₂ y R_{1-133'}, respectivamente. H₂O: Control negativo (reacción de PCR conteniendo todos los componentes menos el ADN molde de la planta en estudio). Las flechas indican ausencia o presencia de bandas.

Figure 1. Detection of genetic variability using ISSR *in vitro* regenerated buffelgrass plants. Agarose gel electrophoresis (2 %) of the PCR product obtained with the following ISSR: (A) 17899, (B) RAF4, (C) UBC827, and (D) UBC834.

Figura 1. Detección de variabilidad genética utilizando ISSR en plantas regeneradas *in vitro* de buffelgrass. Electroforesis en gel de agarosa al 2 % del producto de PCR obtenido con los siguientes ISSR: (A) 17899, (B) RAF4, (C) UBC827 y (D) UBC834.

The results obtained from the sqrt (1-S) transformation of Jaccard's similarity index indicates that the distance coefficients vary between 0.05 and 0.24 (5 and 24%, respectively), between *in vitro* regenerated plants and control (table 2).

The analysis including only the coefficients of the *in vitro* regenerated plants showed values ranging between 0.11 and 0.29 (11 and 29%, respectively), indicating not only divergence from RN 51 but also differences between *in vitro* regenerated plants.

The results of the principal coordinate analysis (PCoA) show that the most important principal components (PCs) (PC 1 and PC 2) (figure 2, page 8), which account for almost 62% of the total variability, shows

two groups; one of them is composed of a single genotype (R_{1-t31}), which showed the greatest differences among the *in vitro* regenerated materials in PC 1. In turn, PC 2, differentiates two groups, one of them including the material used as anther donor or control (C) and the other including two regenerants that were very different from the rest (R_{1-t32} and R_{1-t33}).

Figure 3 (page 8) showed the results of the dendrogram generated by UPGMA clustering method. The former cophenetic analysis yielded a correlation coefficient of 0.97. These results further highlight the differences compared with PCoA, because if considering a distance of 0.13 as an arbitrary cut-off value (50% of the maximum distance), R_{1-t32} and R_{1-t33} individuals not formed a group and the R_{1-t4} individual was separated from the group that included RN 51 (C).

Table 2. Distance matrix of six *in vitro* regenerated buffelgrass plants and genotype RN 51 (C), obtained from the transformation sqrt (1-S) of the Jaccard's similarity index based on 22 ISSR primers.

Tabla 2. Matriz de distancia de seis plantas regeneradas *in vitro* de buffelgrass y del genotipo NR 51 (C), obtenida de la transformación sqrt (1-S) del índice de similitud de Jaccard basado en 22 cebadores ISSR.

	C	R_{1-t31}	R_{1-t32}	R_{1-t33}	R_{1-t4}	R_{1-t5}	R_{1-t6}
C	0						
R_{1-t31}	0.24	0					
R_{1-t32}	0.18	0.29	0				
R_{1-t33}	0.18	0.29	0.22	0			
R_{1-t4}	0.14	0.27	0.21	0.21	0		
R_{1-t5}	0.12	0.25	0.19	0.2	0.16	0	
R_{1-t6}	0.05	0.23	0.18	0.17	0.13	0.11	0

C: Apomictic genotype used as anther donor control plant. R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} and R_{1-t33} : *in vitro* regenerated buffelgrass plants.

C: Genotipo apomítico utilizado como planta control dadora de anteras. R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} y R_{1-t33} : plantas regeneradas *in vitro* de buffelgrass.

Phenotype evaluation

Of the six plants that were transplanted to the field (R_1), five exhibited very different phenotypes from RN 51, and the remaining one did not survive. Plants R_{1-t5} and R_{1-t6} , whose DNA content increased (6), showed significant differences ($P < 0.05$) in four morphological variables: plant height (PH), internode length (INTL), stem width (SW) and number of branches (NBR) (table 3, page 9).

The lower INTL, without modifying the number of nodes (NN), gives these plants a stunt appearance (lower PH). Moreover, the plants have a lower number of branches (NBR). The material R_{1-t5} exhibits scarce panicles and the other one (R_{1-t6}) has a lower panicle length (PanL) ($P < 0.05$). Both regenerants produce few seeds (data not shown). R_{1-t6} also presented a decrease in flag leaf length (FLL), flag leaf width (FLW) and flag leaf sheath length (FSL) ($P < 0.05$).

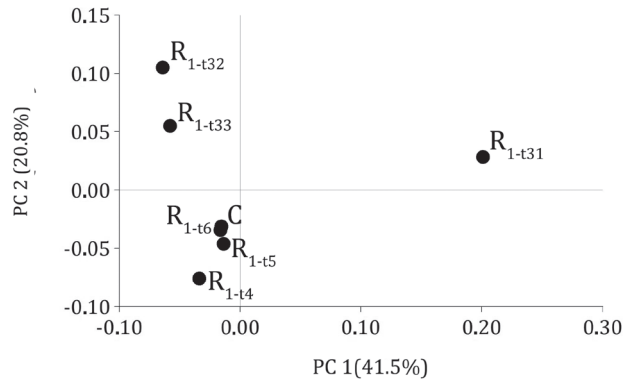


Figure 2. Biplot of six *in vitro* regenerated buffelgrass plants (R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} and R_{1-t33}) and RN 51 genotype used as control (C), obtained from sqrt (1-S) transformation of Jaccard's similarity index based on 22 ISSR primers.

Figura 2. Biplot de seis plantas regeneradas *in vitro* de buffelgrass (R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} y R_{1-t33}) y del genotipo NR 51 utilizado como control (C), obtenido de la transformación sqrt (1-S) del índice de similitud de Jaccard basado en 22 cebadores ISSR.

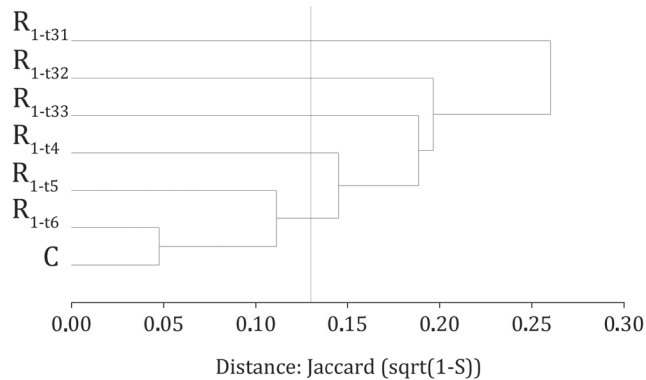


Figure 3. Dendrogram of six *in vitro* regenerated buffelgrass plants (R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} and R_{1-t33}) and RN 51 genotype used as control (C), obtained via UPGMA method through the analysis of ISSR markers over a distance matrix calculated from the sqrt (1-S) transformation of the Jaccard's similarity index.

Figura 3. Dendrograma de seis plantas regeneradas *in vitro* de buffelgrass (R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} y R_{1-t33}) y del genotipo NR 51 utilizado como control (C), obtenido por el método UPGMA mediante el análisis de marcadores ISSR, sobre una matriz de distancia calculada de la transformación sqrt (1-S) del índice de similitud de Jaccard.

Table 3. Characters of external morphology of leaf, stem and panicle analyzed in 5 *in vitro* regenerated buffelgrass plants.**Tabla 3.** Caracteres de morfología externa de hoja, tallo y panoja analizados en 5 plantas regeneradas *in vitro* de buffelgrass.

Material	FLL cm	FLW cm	FSL cm	PH cm	PanL cm	TFPL cm	EPL cm	INTL cm	SW mm	NN	NBR	FL/ BR
RN 51	33.3	1.2	7.2	126.8	11.9	19.4	8.3	10.8	3.9	9.8	6.8	2.4
R _{1-t32}	31.0	1.3	6.2	102.5*	7.75*	15*	5.6	6.02*	3.2	9.2	6.2	2.8
R _{1-t33}	-	-	-	105.8*	-	-	-	6.33*	2.7	12.5	7.7	-
R _{1-t4}	28.7	0.9*	6.4	92.8*	8.5*	17.5	8.7	7*	2.4*	9.6	5.6	3.4
R _{1-t5}	-	-	-	57.4*	-	-	-	4*	2.1*	10.0	2.8*	-
R _{1-t6}	22.4*	0.7*	3.2*	64.2*	6.3*	15.2	6.4	4.5*	2.1*	8.4	3.6*	1.0

(*) Indicates significant differences ($P < 0.05$) within each column in the morphometric characters analyzed in *in vitro* regenerated materials with respect to the anther donor plant (RN 51). Material: *in vitro* regenerated R_i plants. Morphological variables: flag leaf length (FLL), flag leaf width (FLW), flag leaf sheath length (FSL), plant height (PH), panicle length (PanL), total floral peduncle length (TFPL), exerted peduncle length (EPL), internode length (INTL), stem width (SW), number of nodes (NN), number of branches (NBR) and number of flowers/branch (FL/BR).

(*) Indica diferencias significativas ($P < 0,05$) dentro de cada columna en los caracteres morfológicos analizados de los materiales regenerados *in vitro* con respecto a la planta dadora de anteras (NR 51). Material: plantas R_i regeneradas *in vitro*. Variables morfológicas: longitud de lámina de hoja bandera (FLL), ancho de lámina de hoja bandera (FLW), longitud de vaina (FSL), altura de planta (PH), longitud de inflorescencia (PanL), longitud total del pedúnculo floral (TFPL), longitud del pedúnculo excerto (EPL), longitud inter-nodal (INTL), ancho tallo (SW), número de nudos (NUD), número de ramas (RAM) y número de flores/ramas (FL/RA).

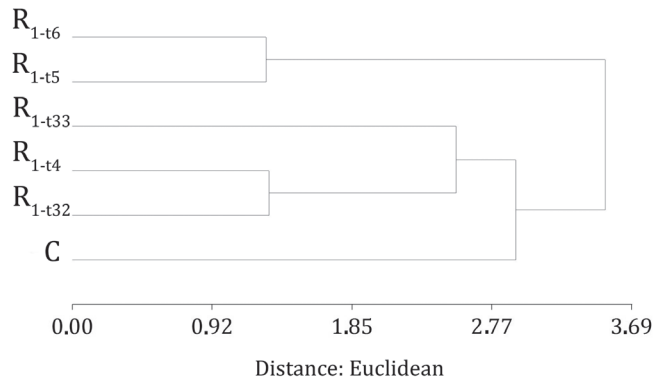
The plants exhibiting a reduction in DNA content (R_{1-t4}, R_{1-t32} and R_{1-t33}) (8) also showed a statistically different increase in phenotypic traits with respect to control ($P < 0.05$) (table 3). All the plants exhibited a decrease in PH. Plant R_{1-t4} had reduced FLW (thinner leaves), as well as PanL, INTL and SW. Plant R_{1-t32} also had smaller panicles, and smaller length of exerted peduncle (EPL) and INTL. Plant R_{1-t4} exhibited particularly lighter-colored foliage.

Because R_{1-t33} and R_{1-t5} plants had few panicles with immature inflorescences, PanL, TEPL, EPL and flowers/branch (FL/BR) variables and characters of leaf (FLL, FLW, FSL) were not considered in the analysis of clusters. The results of this analysis show

that *in vitro* regenerated plants are phenotypically different from genotype RN 51. The dendrogram shows that the group including regenerants R_{1-t32}, R_{1-t4} and R_{1-t33} are the most similar to material RN 51, and that R_{1-t5} and R_{1-t6} are the most different materials (figure 4, page 10).

DISCUSSION

Different molecular profiles were detected in *in vitro* regenerated buffelgrass plants using ISSR molecular markers, suggesting some degree of variability (16, 17, 34). There is scarce information available on the use of this type of markers in somaclonal variation in buffelgrass.



In vitro regenerated buffelgrass plants: R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} and R_{1-t33} . The genotype RN 51 was used as control (C). Cophenetic correlation = 0.938.

Plantas regeneradas *in vitro* de buffelgrass: R_{1-t4} , R_{1-t5} , R_{1-t6} , R_{1-t31} , R_{1-t32} y R_{1-t33} . El genotipo NR 51 fue utilizado como control (C). Correlación cofenética = 0,938.

Figure 4. Dendrogram constructed using UPGMA method through the analysis of morphological markers, with Euclidean distance being applied as proximity measure.

Figura 4. Dendrograma construido utilizando el método UPGMA mediante el análisis de marcadores morfológicos, aplicando la distancia Euclídea como medida de proximidad.

Studies conducted with ISSR in other *in vitro* propagated species reported very different polymorphic levels. For example, a 50% polymorphism level was detected in variants of *Camellia sinensis* L. (30); lower levels, of 5 and 3.9%, were recorded in micro-propagations of *Musa* spp. and *Dictyospermum ovalifolium*, respectively (10, 26). Here, total polymorphic loci were found to be 12%, which is similar to results reported for *Codonopsis lanceolata* (15.7%) or in clones of *Camellia* spp. (12.8%) (12, 15).

Likewise, the generated polymorphism showed a differentiation degree of 5 to 24% with respect to the "control" pattern, as observed in somaclones of *Zea mays* L. (24). In addition, it is important to note that using the same type of molecular marker similar levels of polymorphism were obtained in wild populations of buffelgrass (1).

Amplification products were observed with the primers containing di-, tri- or tetra-nucleotide sequences, with or without additional nucleotides at the 3' end. While most of the ISSR produced bands using the method implemented here, some of them failed to amplify. These primers were then tested at lower annealing temperatures, since molecular patterns were found to be highly modified by this factor (14), but some of these ISSR also failed to amplify. Lower temperatures could be used (16) or other additional factors should be considered, which were not studied in this work (5, 34).

The most informative primers were those containing repetitive sequences $GACA_n$, AG_n or GA_n , with the addition of one or three nucleotides at the 3' end. Similar results were described in a study conducted to determine genetic diversity in wild buffelgrass populations (17).

Hence, this results confirm that the motifs (GA)_n and anchors at the 3' end can be included in the ISSR markers used for different studies of this species.

The polymorphism detected by ISSR in *in vitro* regenerated buffelgrass plants may be attributed to genetic changes in a restricted portion of the genome, because microsatellites and their continuous bases are the hybridization site of the marker primer (16, 34). This portion of the genome usually undergoes changes during the DNA replication-repair process (22, 29), and some authors suggest that the generated polymorphism may be due to deletions or insertions that occur at the primer recognition site (24). Regardless of the type of mutations occurring during *in vitro* plant regeneration, they can generate gain or loss of alleles (28), as shown in this work.

Finally, plants transplanted to the field exhibited very different morphological characteristics among them and from the genetic material used as control (RN 51), thereby confirming changes in the phenotype.

This study, along with a previous one (8), clearly reveal that a group of *in vitro* regenerated plants undergo genetic and morphological changes with respect to the anther donor material, suggesting that *in vitro* culture technique is a source of somaclonal variation (20).

These observations suggest that, besides the changes detected by flow cytometry at the nuclear DNA content levels (8), *in vitro* regenerated plants might also have undergone specific mutations in the genome (24). It is possible that these changes restricted to a few nucleotides cannot be detected using flow cytometry. For this reason, ISSR molecular markers should be included to explore genetic variations in *in vitro* regenerated buffelgrass plants.

CONCLUSIONS

The use of ISSR allowed to identify genetic variability in *in vitro* regenerated buffelgrass plants.

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Absorción de agua de hidrogel de uso agrícola y su humedecimiento de tres tipos de suelo

Water absorption hydrogel agricultural use and wetting of three soil types

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Nota científica

RESUMEN

El objetivo fue evaluar el porcentaje de absorción de agua y su humedecimiento en tres tipos de suelo. Se utilizó como material experimental al Polímero de Acrilamina de Potasio (PAP) y tres suelos de tipo arenoso, limoso y arcilloso. Se midió la cantidad de agua (mL) y el tiempo (minutos) que absorbe 1 g de PAP. Además, se evaluó el humedecimiento de PAP para cada tipo de suelo colocando 1 g en suelo seco, previamente se midió la humedad del suelo antes y después de colocar el PAP. Los resultados indican que el hidrogel PAP absorbe por cada gramo alrededor de 103 mL, dicha absorción va en aumento en función del tiempo llegado a un punto de inflexión a los 60 min, posterior a este tiempo el aumento no es significativo. El humedecimiento tuvo un aumento variable en función del tipo de suelo, siendo 14,7, 17,4 y 14,5% para los suelos arenoso, limoso y arcilloso respectivamente. Este humedecimiento se obtiene después de alrededor de una hora. En suelo saturado los geles permanecen sin modificaciones. En cambio en un suelo seco, los geles ceden el agua contenida al suelo, pasando a ser parte del contenido de agua del suelo.

Palabras clave

absorción • humedecimiento • hidrogel PAP • suelo arenoso

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ABSTRACT

The objective was to evaluate the percentage of water absorption and wetting in three soil types. It was used as experimental material to Acrylamide Polymer Potassium (PAP) and three floors of sand, loam and clay type. The amount of water (mL) and time (minutes) which absorbs 1g of PAP was measured. In addition, the wetting of PAP for each soil type by placing 1 g dry soil was evaluated previously soil moisture was measured before and after placing the PAP. The results indicate that the hydrogel absorbed per gram PAP about 103 mL, this absorption is increasing function of time reached, after this time turning point at 60 min the increase is not significant. It wetting had a variable increase depending on the type of soil, being 14.7, 17.4 and 14.5% for sand, loam and clay soils respectively. This wetting is obtained after about an hour. In saturated soil gels remain unchanged. However in a dry soil, the gels gives the contained water to the ground becoming part of the soil's water.

Keywords

absorption • wetting • hydrogel PAP • sandy soil

INTRODUCCIÓN

Cada vez se hace más intensivo el cuidado por el agua dulce en el mundo, por lo que se realizan investigaciones de cómo ser más eficiente en su uso en especial en la agricultura, ya que este sector es la que más consume agua, más aún si existe escasez, como es el caso de la zona centro norte de la provincia de Manabí. Donde se está utilizando como alternativa productos como los polímeros hidrorretenedores que hacen eficiente el uso del agua.

Sin embargo, no se está evaluando en función de las características del suelo, en este sentido Ruiz *et al.* (2016) mencionan que la retención de humedad está relacionada con la superficie específica y el contenido de arcilla. Este hecho puede provocar un mal uso del producto, que podría repercutir en el suelo e incluso en los cultivos. Se dan recomendaciones técnicas de uso, pero estas son muy generalizadas y no se pueden adaptar a todos los tipos de suelo.

Las Acrilamidas es uno de los monómeros utilizados para sintetizar los

hidrogeles para aplicaciones hortícolas (1) y su eficacia se describe en distintas publicaciones desde los años 80 (13, 16, 17), donde se cita una variedad de usos en la agricultura.

En la explotación forestal al momento de la siembra, es muy útil usar polímeros sintéticos aplicados como auxiliar en el manejo de la plantación y para proteger las raíces, cuya principal característica es su alta capacidad de retención de agua (7, 9). Esto permite incrementar la frecuencia de riego, y disminuir las pérdidas de productos químicos por lixiviación y lavado (13).

En otro ámbito, es usual utilizarlo en viveros donde es común el uso de arena como sustrato, o en lugares donde existe producción agrícola con un suelo de tipo arenoso. Idrobo *et al.* (2010) probaron la aplicación de hidrogel en suelo arenoso en el cultivo de rábano, señalando que la mayor cantidad de hidrogel en un suelo incrementa la eficiencia en la retención de la humedad.

Santelices (2005) lo recomienda para el establecimiento de *Eucalyptus globulus* ya que minimiza la mortandad por estrés hídrico.

Las propiedades de los hidrogeles presentan algunas dificultades de usos agrícolas como horticultura. Uno de ellos es la fuerza que ejerce el suelo para poder absorber el agua de los geles, por otro lado, la cantidad de agua que pueden retener los geles.

Estudios previos han demostrado que la cantidad de agua absorbida disminuye por las propiedades mecánicas del suelo (15). Sin embargo, todos los polímeros hidroretenedores presentan su principal beneficio en absorber agua y luego cederla a las plantas lentamente (10).

Vlach (1991), indica un aumento de 100 veces su peso, para después liberarla en función de las necesidades de la planta. Con estos antecedentes se planteó como objetivo evaluar la absorción de agua y el humedecimiento del hidrogel en tres tipos de suelo, con la hipótesis de que el hidrogel se comporta diferente en función de la textura del suelo.

MATERIALES Y MÉTODOS

La investigación se llevó a cabo en el laboratorio del área productiva de la Universidad Laica Eloy Alfaro de Manabí Extensión Chone-Ecuador. Se estudió la cantidad de agua (mL) que absorbe el polímero de poliácralamina de potasio (g) y su propiedad para humedecer suelos de diferentes clases texturales.

Manejo del experimento

El experimento se condujo en dos fases, en la primera se estudió la cantidad de 1 y 2 g de hidrogel en las muestras de cada tipo de suelo. Se distribuyeron en un diseño completamente al azar y se aplicó la cantidad de 1 y 2 g de hidrogel y el volumen de agua según corresponda a cada tratamiento en estudio.

Absorción de agua del hidrogel

La absorción del hidrogel consistió en medir la cantidad de agua que retiene. Para lo cual se realizaron pesadas de 1 y 2 gramos de hidrogel y se le aplicó 100 y 200 mL para 1 g, y 200 y 300 mL para 2 g de hidrogel. Se realizaron tres repeticiones de cada tratamiento. Una vez iniciada la hidratación, se midió la cantidad de agua retenida haciendo drenar el agua no retenida y restándola de la cantidad inicial. El tiempo de hidratación fue de 20, 30, 60, 120 y 150 minutos.

Humedecimiento del hidrogel

Se tomaron tres tipos de suelo con diferencias, texturas: arenoso, limoso y arcilloso. Se tomó muestra de cada suelo y se dejó secar a la sombra. Posteriormente se colocaron en recipientes de 20 cm de diámetro y 40 cm de alto. Se realizaron tres repeticiones de cada tratamiento. En cada recipiente se colocó en el centro del recipiente 1 g de hidrogel hidratado. Previa a la aplicación del hidrogel se midió la humedad por gravimetría.

Después de 24h se tomó muestras del suelo y se determinó su humedad por gravimetría y así establecer el aumento de humedad de cada suelo. Los datos se tabularon y analizaron mediante estadística descriptiva, con los estadígrafos, media y desviación estándar.

RESULTADOS Y DISCUSIÓN

Evaluación de la absorción del hidrogel

El polímero de poliácridamina tuvo un límite de absorción de agua de $103,25 \pm 6,8$ mL (tabla 1) en un tiempo de una hora. En un tiempo menor la absorción es considerablemente menor, y en mayor tiempo la absorción es ligeramente superior a lo cual se consideró como depreciable. Con estos datos se pudo considerar que el tiempo óptimo de hidratación es de una hora y que en ese tiempo cada unidad de hidrogel absorbe 103 mL. En la figura 1, se observa la tendencia entre la cantidad de agua absorbida y el tiempo.

Los resultados indican diferencia respecto de la descripción del producto, el cual hace referencia de un aumento hasta 300 veces su peso.

En este caso al considerar volumen se podría decir que llegó a 100 veces su peso ya que por cada gramo absorbe 100 mL de agua (cada gramo equivale a un mL de agua). Se debería proseguir las investigaciones para determinar cuál es la causa de dicha diferencia.

Tabla 1. Valores de agua absorbida del hidrogel en distintos tiempos.

Table 1. Values of the hydrogel of water absorbed at different times.

Tiempo (minuto)	Agua Absorbida (mL/g)
20	50 ± 5
30	$65 \pm 2,5$
60	$103,25 \pm 6,8$
120	$103,4 \pm 14,2$
150	$107,4 \pm 11,6$

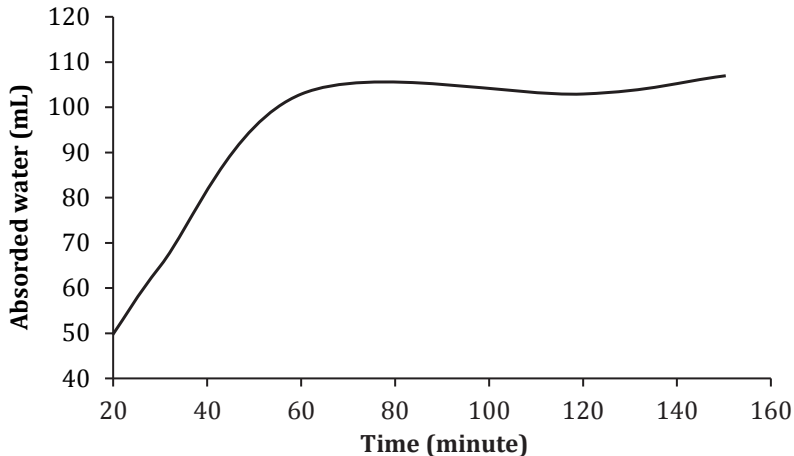


Figura 1. Tendencia de absorción de agua del hidrogel en el tiempo.

Figure 1. Trend water absorption of the hydrogel over time.

Sin embargo, comercialmente se sabe que las empresas que proveen el hidrogel sobreestiman sus propiedades. Otro aspecto a tomar en cuenta es el tipo de agua a utilizar, en este sentido Bowman (1990) en ensayos realizados con agua desionizada y agua con $0,5 \text{ ds.m}^{-1}$ de conductividad eléctrica, encontraron que se alcanzaba un máximo de 420 g de retención por cada g de hidrogel en el agua desionizada y en el caso del agua con $0,5 \text{ ds.m}^{-1}$, era ligeramente superior a los 100 g, similar al resultado encontrado en este estudio.

Arbona *et al* (2005), presentan un aumento de peso de alrededor de 30% al usar una concentración de 0,4% de hidroretenedor; frente a un sustrato tipo enmienda en día cero y en el día 21 presentaron un porcentaje superior al 60%. El porcentaje de retención de agua suele estar dado por la composición del producto. Así Martyn y Szot (2001) encontraron diferencia al comparar dos productos: el Alcosorb-400 y Akrygel-RP al ser mezclado con diferentes sustratos. Por otro lado, Bres y Weston (1993) encontraron un porcentaje de retención superior a 300% en dos diferentes hidroretenedores en estudio realizados con la aplicación de fertilizantes. Sobre la base de esta información se puede concluir que existe la necesidad de corroborar los datos encontrados hasta el momento en esta investigación y evaluar otros factores que

podieran estar influyendo en la retención de agua, lo que permitirá tener una idea clara antes de recomendar su uso en la zona de producción agrícola de la región.

El hidrogel se comporta diferente dependiendo de la textura del suelo. En suelos de características arenosa aumenta un 14,7% la humedad, en suelo con textura limosa 17,4% y en suelos pesados o arcillosos un 14,5% (tabla 2). Los aumentos de humedad son realmente considerables, este fenómeno no se observa cuando el suelo está saturado o en capacidad de campo. Se pudo observar también que el gel prácticamente desaparece al colocarlo en suelo seco, y permanece sin modificarse en suelos saturados.

Los hidroretenedores deben tener la capacidad de una fácil absorción de agua pero también de facilidad para entregar agua. Esta entrega de agua al parecer tiene relación directa con el tipo de suelo o sustrato, puesto que no sería lo mismo proveer de agua en un suelo arenoso que un suelo arcilloso y con cualquier otro tipo de suelo, debido a las diferencias de las propiedades físicas. En este sentido Martyn y Szot (2001), presentan valores diferentes al aplicar hidrogel en diferentes sustratos, muestran que con turba la retención de agua es superior que al ser aplicada en sustrato de mezcla de turba, con corteza de árboles o perlita.

Tabla 2. Humedecimiento del hidrogel en suelo de diferentes texturas.

Table 2. Wetting of the hydrogel in different soil textures.

Tipo de suelo	Humedad seco (%)	Humedad hidratado (%)*	Incremento Promedio (%)
Arenoso	8	22,7±0,6 b	14,7
Limoso	14	31,4±1,15 a	17,4
Arcillo	17	31,5±0,9 a	14,5

a, b: letras distintas en una misma columna indican diferencias estadísticas según Tukey 0,05. * Probabilidad estadística $P < 0,01$.

a, b: different letters in the same column indicate statistical differences according to Tukey 0.05. * Statistic probability $P < 0.01$.

Otro parámetro observado pero no medido es el área a hidratar, el cual probablemente esté relacionado con la cantidad de hidrogel que se aplique y el tipo de suelo. Por esta razón se necesita realizar investigaciones dirigidas para medir el área a humedecer en función de la cantidad de hidrogel y para cada tipo de suelo. Esto sin duda permitirá establecer la forma de aplicación en el suelo. Por el momento los resultados preliminares indican diferencia de entrega de agua debido a la textura del suelo.

CONCLUSIONES

El hidrogel almacena alrededor de 103 mL de agua por cada gramo de hidrogel. El tiempo necesario para que el hidrogel almacene el agua es de una hora como mínimo. Además, existe una relación directa entre el tipo de suelo principalmente por su textura y la facilidad que tiene el hidrogel hidratado en ceder agua al suelo.

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Hydrogel for improving water use efficiency of *Capsicum annuum* crops in Fluvisol soil

Eficiencia del agua de riego mediante la aplicación de hidrogel en *Capsicum annuum* en suelo Fluvisol

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ABSTRACT

This study aimed at assessing the effect of hydrogel on irrigation water use efficiency and yield of *Capsicum annuum* crops. It was used the hybrid pepper variety El Salvador as the experimental material, with row widths of 1.0 m and a 0.5 m separation between plants. It was evaluated five pre-hydrated gel doses: 0.5, 1.0, 1.5, 2.0 and 2.5 g/plant. Treatments were arranged in a randomized complete block design. The variables under study were: water consumption, irrigation frequency and water use efficiency, plant height, fruit characteristics, and yield. Results showed that hydrogel, at doses ranging from 2 to 2.5 g/plant, reduced the depth of application from 388.6 mm to 197.6 and 196 mm, respectively. Water efficiency was correlated with hydrogel use producing up to 10.1 kg.m⁻³, whereas the control treatment reached 5.1 kg.m⁻³. Production variables did not show statistical significance. The conclusion is that hydrogel worked as a water retainer releasing water into the Fluvisol soil and making water use more efficient in pepper crops, without affecting plant development.

Keywords

water retainer • water consumption • irrigation frequency • yield of pepper crops

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RESUMEN

El objetivo fue evaluar la influencia de la dosis de hidrogel en la eficiencia de riego y en el rendimiento del cultivo de pimienta. Se utilizó como material experimental el híbrido Salvador el mismo que se estableció a un distanciamiento de 1,0 m entre hileras y 0,5 entre plantas. Se estudiaron cinco dosis: 0,5; 1,0; 1,5; 2,0 y 2,5 g/planta, el mismo que fue previamente hidratado. Los tratamientos se establecieron en un diseño de bloques completos al azar. Se midieron las variables relacionadas con el riego: consumo de agua, frecuencia de riego y eficiencia del uso del agua; además, la altura de planta, características del fruto y el rendimiento. Los resultados indican que la aplicación del hidrogel con una dosis entre 2 y 2,5 g/planta se obtiene una reducción de la lámina de aplicación de 388,6 mm a 197,6 y 196 mm respectivamente. La eficiencia del agua tiene relación con el hidrogel produciendo hasta 10,1 kg.m⁻³ a diferencia del testigo que alcanzó 5,1 kg.m⁻³. Las variables productivas no presentaron influencia estadística. Se puede concluir que el uso de hidrogel actúa como un retenedor de agua que la libera fácilmente en un suelo Fluvisol haciendo eficiente el uso del agua en un cultivo de pimienta sin afectar su desarrollo.

Palabras clave

retención de agua • consumo de agua • frecuencia de riego • rendimiento del pimienta

INTRODUCTION

In Ecuador, there is a broad supply of water retainers or conditioners for increasing water retention in agricultural soils. However, they are generally used without a proper consideration of soil and crop types. This has perhaps limited the use of this technology among local farmers. The use of water retainers is still very sporadic despite their broad spectrum of benefits; probably due to the lack of scientific information from local research concerning its use. In the province of Manabí, and mainly in places where there is difficulty to access water, farmers use hydrogel on horticultural and perennial crops, but without considering any formal instructions on its application, dose and soil type.

Buck and Evans (2010) found that hydrogel could modify the physical properties of soils (*i.e.* apparent and real densities, pore spaces, and water retention capacity). The changes were mainly asso-

ciated with hydrogel dose. The modifications also occurred after adding other soil improvers, for example peat, for this reason hydrogel is also known as a soil conditioner. Martyn and Szot (2001), after evaluating two commercially available hydrogel types, at different concentrations in soil, found variations, though without any clear trend. The same response was produced by other organic components such as peat and vermiculite. Rivera *et al.* (2015) indicate that hydrogel has an effect on the time the soil needs to dry up. On the other hand, when used in dry soil, hydrogel can moisten the soil in levels that vary depending on soil type. Arbona *et al.* (2005) reported an increase of 30% in soil moisture after using hydrogel. Authors such as Johnson and Leah (1990) and Nissen (1995) have commented on the importance of using hydrogel on forest trees, especially during the sowing period.

When water is scarce, it results in massive loss of crops. Thus, hydrogel can be used as a solution to prevent such losses. Save *et al.* (1995) recognize that hydrogel can decrease the frequency of irrigation and reduce water lixiviation. According to Santelices (2005), in *Eucalyptus goblulus*, harvested in the spring, hydrogel contributed to the survival of plants with less access to water. On this ground, the aim was to assess the effect of hydrogel on water use efficiency of *Capsicum annuum* crops in Fluvisol soil.

MATERIALS AND METHODS

The study was conducted in the Carrizal river valley, in the municipality of Bolívar, Manabí, Ecuador. The valley is typically formed by Fluvisol soil as a consequence of previous floods. The area is geographically located between 0° 39' 34", South Latitude; and 80° 02' 45.07", West Longitude; at an altitude of 16 meters above sea level.

It was evaluated 5 doses of agricultural hydrogel, comprised of 1% potassium polyacrylamide, which were added to the soil at the base of each plant. The doses were: 0.5 (T1), 1.0 (T2), 1.5 (T3), 2.0 (T4) and 2.5 (T5) g/plant, plus a control treatment consisting of a plot without hydrogel.

Soil characteristics

The soil was primarily composed of Fluvisol, which is formed by sedimentation layers (5). Its corresponding texture was 70% sand, 8% silt and 22% clay. The field capacity was 0.23 cm³ (H₂O)/cm³ (soil), with a total tension of 10 centibars and an apparent density of 1.3 g/cm³.

Plant material

The hybrid pepper variety El Salvador was used as the plant material, with row

widths of 1.0 m and a 0.5 m separation between plants. Seeds were placed in germinating trays for transplant after 21 days.

Experimental design

A randomized complete block design. Was used with four replicates for each treatment. Each experimental unit consisted of a 24 m² plot, with 48 plants distributed along three drip irrigation tubes of a 1.0 m separation and 32 m long. Each treatment was replicated four times.

Hydration

Following the indications of Rivera *et al.* (2015), hydrogel was hydrated before being added to the soil, using 100 ml of water/g for one hour. It was then placed in the soil at a depth of 25 cm at each plant.

Irrigation

The drip irrigation system consisted of an irrigation head, a filter, a pressure gauge and a valve. The drip irrigation tubes had a valve for controlling the flow for each treatment. The lateral line had a diameter of 50 mm, and the drip tubing was of 16 mm in diameter, releasing a flow of 4 L/h.

Irrigation was programmed through tensiometers, which were used for each treatment. First, data on tension and soil moisture were collected, and then the values were adjusted to fit the equation by Van Genuchten (1980).

$$\theta = \theta_r + \left(\frac{\theta_s - \theta_r}{[1 + (\alpha h)^n]^{\frac{1}{n-1}}} \right)$$

where:

θ = moisture content (at a specific tension)

θ_r = residual moisture content

θ_s = saturation moisture content

h = soil tension

α = optimized parameter

n = optimized parameter

Field capacity (FC) was 10 centibars. Irrigation time was programmed at 20 centibars or above. After data collection, it was used the replacement depth equation as follows:

$$Lb = (CC - HA) \times da \times D \times Fai$$

where:

Lb = raw sheet replacement

CC = field capacity

HA = moisture content during irrigation

da = apparent soil density

D = soil stratum depth

Fai = adaptation factor

After calculating the depth of irrigation, it was calculated the irrigation time using the following equation:

$$Tr = \text{raw volume} / \text{dripping flow rate}$$

Analyzed parameters

Variables related to irrigation

It was considered water consumption (mm), taken from the sum of the irrigation depths used for each irrigation; the frequency, as the interval of days between irrigations; and the total number of irrigations. Water efficiency was obtained from the relationship between consumption in m³ and kilograms of pepper produced.

Plant height

The height (cm) was measured from the base of the plant up to the terminal apex during days 7, 14, 21, 40, 60, 80 and 100 after transplant, using a measuring tape.

Fruit characteristics

Among these variables, was considered: length, weight and diameter. Length and diameter were measured in centimeters using a Vernier caliper. Fruit weight was measured in grams using an analytical laboratory scale.

Yield

As the sum of total harvest weight per unit of area, expressed as t/ha. The total number of fruits per plant was obtained from the sum of the fruits produced from the harvest divided by the number of plants.

Statistical Analysis

Using irrigation variable data, was created average frequency tables showing total water consumption for each treatment. The other variables were evaluated through analysis of variance and the Tukey test at the 5% level of probability.

RESULTS AND DISCUSSION

Data regarding irrigation variables are presented in table 1 (page 27). Treatments showed big numerical differences mainly in water consumption. The differences were more notorious in T4 and T5, with values reaching total consumption of 197.6 y 196 mm, respectively.

The control treatment (without hydrogel) reached a consumption of 388.6 mm, showing that it was possible to save up to 40% water. With this respect, Agaba *et al.* (2011) found that hydrogel, at 0.4%, reduced at least half the water consumption of the control treatment in a sandy soil. In contrast to Montesano *et al.* (2015), who found that the application of hydrogel, at 2%, significantly increased moisture retention in sandy soil; in this type of soil, hydrogel tends to be more efficient than in clay soils. López *et al.* (2013), while evaluating a polyacrylamide hydrophilic polymer on Anaheim chili, found that it reduced the volume of water, though not down to the level shown in this study. This may be due to the fact that, in this research, the polymer was placed at the base of each plant, which proved to be more efficient.

Table 1. The six treatments and variables related to irrigation.**Tabla 1.** Variables relacionadas con el riego en los tratamientos en estudio

Treatments	Water use (mm)	Irrigation frequency (days)	# of irrigations	Water efficiency (*Kg.m ⁻³)
T1	376.1	2 y 3	44	5.5
T2	374.4	2 y 3	44	5.3
T3	301.6	2 y 4	36	6.7
T4	197.6	3 y 6	22	9.9
T5	196	3 y 6	22	10.1
Control	388.6	2 y 3	44	5.1

* Kilogram of pepper produced per cubic meter of water.

* Kilogramo de pimienta producido por metro cúbico de agua.

Previous research has used a mix of soil substrates in pots (5, 6, 10, 13), but when used in the field, during normal plant development, it may be possible to obtain different results.

Irrigation frequency varied from 2 to 6 days depending on the treatment. This happened because the frequency was not pre-established, but was subjected to soil tension, which was influenced by the effect of hydrogel. Treatments with more hydrogel (T4 and T5) reported up to a six-day irrigation interval. Treatment T1, T2 and Control had a frequency of 2 and 3 days, respectively. T3 had a frequency ranging from 2 to 4 days. This frequency increase made the number of irrigations smaller, thus less water was needed. Wadas *et al.* (2010), from experiments carried out in *Eucalyptus urograndis* plantations, argue that moderated stress symptoms, due to water deficit, can last up to ten days.

The obtained water use efficiency suggests that the application of hydrogel, at doses between 2.0 y 2.5 g/plant, can produce up to 10.1 kg.m⁻³ of *C. annum* showing higher values than other treatments. López *et al.* (2013), while evaluating a water-absorbing polymer, at a dose of 25 kg.ha⁻¹ added to a sandy-loam soil, did not find any statistically significant differences.

Hydrogel is considered to be an essential element for saving water in pepper crops, without affecting its productivity.

Hydrogel particles retain water, and then slowly release it into the soil when the plant needs it. Water encapsulation inside the hydrogel particle prevents losses due to evaporation, intensifying the effect when the particle is in the soil. However, the stress caused by dry soil makes hydrogel particles release the encapsulated water. On the other hand, Rivera Fernández *et al.* (2018) comment on the relationship between soil texture and water conveyance by hydrogel. Also, the specific soil particle surface has been found to affect water retention in soil (18). It is necessary to emphasize that the above-mentioned outcomes occurred when the doses of hydrogel ranged between 2.0 y 2.5 grams per plant; using it in lesser amounts did not produce any effect. Therefore, we can argue that an increase in hydrogel levels makes water retention more efficient, as stated by Idrobo *et al.* (2010), who studied hydrogel in sandy soils.

Plant height

Table 2 (page 28) shows plant height values on the different evaluation days.

Table 2. Average height values of the plants (cm) on different days.**Tabla 2.** Valores promedios de la variable altura de planta (cm) en diferentes días.

Treatments	Plant height (days)						
	7	14	21	40	60	80	100
T1	11.63	20.7	30.8	50.68	70.05	81.8	86.15
T2	11.73	20.43	31.03	50.13	71.13	82.63	84.63
T3	11.5	20.8	31.18	50.98	70.3	79.95	85.58
T4	11.48	20.13	31.63	51.48	70.43	80.9	84.78
T5	11.05	19.88	31.15	49.3	70.95	81.48	84.6
Control	11.73	20.1	30.68	50.38	70.08	80.53	85.75
Probability	0.9	0.87	0.97	0.4	0.53	0.33	0.63
Standard error	0.55	0.61	0.84	0.73	0.49	0.86	0.8

According to the analysis of variance, there was no significant statistical difference ($p>0.05$), which suggests that hydrogel had no negative effect on crops. However, there are numerical differences in each one of the evaluations conducted.

The highest values on day 7 were obtained with the Control and T2, 11.73 cm; followed by T1, 11.63. The smallest value was obtained with T5, 11.05 cm. On day 14, all treatments showed an increase in height, as in the first evaluation. T5 showed the smallest value, 19.88 cm. The other treatments had values above 20 cm. On day 21, there was a similar increase to that of the first evaluations. Height, among

treatments, did not show a trend that suggests a significant difference. The same situation remained until day 100.

Fruit characteristics

Table 3 shows the characteristics of *C. annuum* fruits, with weight and length not presenting any statistically significant difference ($p>0.05$). However, as can be seen, there were differences in fruit diameter ($p=0.002$). With respect to weight, despite not showing statistical differences, it was observed that all treatments exceed 100 g, with T1 having the largest average value, 114.87 g, and T2 the smallest mean value, 106.98 g.

Table 3. Variables related to pepper fruit characteristics.**Tabla 3.** Variables de las características del fruto de pimiento.

Treatments	Fruit variables		
	Fruit weight (g)	Length (cm)	Diameter (cm)
T1	114.87	12.13	5.93 ab
T2	106.98	10.15	5.45 bc
T3	107.93	12.53	6.08 a
T4	107.55	10.13	5.58 abc
T5	108.18	12.63	5.88 abc
Control	109.8	11.43	5.38 c
Probability	0.31	0.36	0.002
Standard error	2.56	1.04	0.11

Different letters in a column indicate statistical difference, Tukey at 0.05.

Letras diferentes en una columna indica diferencias estadísticas, Tukey 0,05.

In the initial harvests, pepper fruits had more weight than at the end of the experiment. Likewise, the variability in fruit characteristics, even from the same treatment, was high. The values presented here are the average results from all harvests, thus they give the impression of having less variability.

Fruit length and weight presented only numerical differences and not any statistical significance. Values ranged from 10.13 to 12.63 cm, T4 and T3, respectively. As with weight, there was a high variability among pepper fruits. Sometimes fruits were of 25 cm in diameter, but other times, they were only 5 cm.

Diameter was affected by the studied variable, showing statistical differences ($p=0.002$). T3 was different from the other treatments obtaining the first statistical category with an average value of 6.08 cm. The smallest diameter was that of the control treatment with 5.38 cm. Despite having statistical difference, variation among treatments is not as notorious as with the previously analyzed variables.

Fruit variables such as length and diameter are important when commercializing the product. Fruit must preferably be between 12-15 cm in size. However, in this study, most fruits were above or below this size, which made fruit classification more difficult. These variables are determined by the characteristics of the plant material. Other variables related to crop management, for example fertilization, irrigation and number of fruits per plant are also influential. There could be plant material with more length, such as Tikal, which was studied by Lesser (2004), reaching 17.88 cm in size. The other material evaluated by the same author ranged between 10.12 y 14.56 cm.

Yield

Yield variables did not show statistical differences ($p>0.05$). The number of fruits

per plant ranged from 8.93 y 9.35, treatments T1 and T2, respectively. The values were obtained from the sum of all harvests.

Yield, in $t\cdot ha^{-1}$, was not affected by hydrogel, presenting similar yield values. This may be due to the fact that all treatments received the required amount of water. Treatment T1 obtained the largest average value $20.53 t\cdot ha^{-1}$, followed by T3 with $20.3 t\cdot ha^{-1}$, and T4 with the smallest mean value of $19.48 t\cdot ha^{-1}$. After obtaining high differences between hybrid and traditional varieties, Lesser (2004) concluded that yield is dependent on plant material.

Table 4. Yield, expressed in number of fruits per plant and tons per hectare.

Tabla 4. Rendimiento en números de frutos por planta y toneladas por hectárea.

Treatments	# fruits/plant	$t\cdot ha^{-1}$
T1	8.93	20.53
T2	9.35	20.03
T3	9.4	20.3
T4	9.05	19.48
T5	9.1	19.7
Control	9.08	19.95
Probability	0.54	0.91
Standard error	0.2	0.72

CONCLUSIONS

Hydrogel, at doses between 2 to 2.5 g/plant, reduced irrigation water consumption making water use more efficient by producing twice as many kilograms of pepper per cubic meter of water.

Hydrogel, when added to Fluvisol soil, increased the water retention capacity, and released water into the soil when needed, without affecting the yield because no difference was found when compared to yield values from the control treatment.

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The phenomenon of cavitation in grapevine... Unravelling implicated mechanisms

El fenómeno de la cavitación en vid... Descifrando los mecanismos implicados

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ABSTRACT

Cavitation is a physiological dysfunction that takes place in the xylem of water stressed plants. It leads to a loss of hydraulic conductance (k_L) as the vessels are filled with air. This impacts water supply, water potential (Ψ_L) and canopy hydration. Stomatal closure is an effective response upon diminishing momentary or seasonal foliar hydraulic contents. Depending on each type of plant, stomata may close preventing catastrophic cavitations. This research intended to understand how stomatal control acts upon cavitation events in two contrasting grapevine varieties, Syrah and Grenache. A mechanistic was developed model based on the water and vapour fluxes, k_L , stomata conductance (g_s), and the vulnerability to cavitation of the xylematic tissue. The theoretical model explains how plants respond to drought and avoid catastrophic cavitation. Water stressed grapevines couple their g_s with their k_L in order to avoid embolism. It is not stomatal closure, by itself, the controlling mechanism. Grapevines under mild water stress, do not need to completely close their stomata in order to avoid cavitation, therefore, photosynthesis is not completely impeded, and the cost in terms of carbon assimilation is less than expected for other species.

Keywords

cavitation • stomatal conductance • hydraulic conductance • mechanistic model • Syrah • Grenache

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RESUMEN

La cavitación es una disfunción fisiológica que ocurre en el xilema de las plantas bajo déficit hídrico, y que entraña una pérdida de su conductancia hidráulica (k_L), cuando algunos vasos se llenan de aire. Esto incide negativamente sobre la oferta de agua y afecta el potencial hídrico foliar (Ψ_L) y la hidratación de la canopia. El cierre estomático es una respuesta efectiva ante la disminución del contenido hídrico. Dependiendo de la especie vegetal, los estomas suelen cerrarse para evitar la cavitación catastrófica. Mediante un modelo mecanístico, que se construyó teniendo en cuenta los flujos de agua y vapor, las k_L y conductancia estomática (g_s), y la vulnerabilidad del xilema a cavitar; se probó que g_s no es la única variable responsable de frenar la embolia. Se determinó que g_s y k_L están íntimamente asociadas y que este acople entre ambas conductancias es lo que frena la embolia. Se concluyó que, en la vid y bajo niveles de estrés hídrico moderado, no es necesario un cierre estomático para controlar la cavitación. Por esto, el mecanismo de control de la cavitación en la vid no conlleva un costo en términos de intercambio gaseoso.

Palabras clave

cavitación • conductancia estomática • conductancia hidráulica • modelo mecanístico • Syrah • Grenache

INTRODUCTION

Drought resistant crops have adaptive physiological and morphological traits that allow them to survive and grow under severe water deficit, resisting dehydration (14, 24). The origin of plant dehydration is embolism formation and catastrophic cavitation. In a dry soil, with low water potential, increasing xylem tension, triggers cavitation. This phenomenon consists on the formation of air bubbles inside the xylem vessels, and subsequently, the breakage of the water column (35). As consequence, the plant suffers a loss of hydraulic conductance (k_L) and desiccation (6, 11, 34, 35). Vulnerability curves relate the percentage loss of plant k_L (PLC) or embolism to the increasing applied pressures that cause that drop of k_L . This pressure may be paralleled to the xylem tension, given that this positive pressure can be considered as equal, but opposite, to the negative pressure inside the xylem (1, 12, 31, 34).

It is well known that stomatal control prevents excessive water loss in an attempt to maintain k_L and prevent desiccation under high evaporative conditions. Several authors have also concluded that stomatal adjustment limits cavitation (4, 8, 15, 19, 21, 28) and that the mechanism is subjected to hydraulic and hydromechanic laws (3, 4, 11). In general, grapevines have been considered as drought avoiding specie due to their efficient stomatal control (8). Most of the water that enters the plant (constituting k_L), leaves through opened stomata (depending on stomatal conductance, g_s) as transpiration (E) (9).

In this sense, many authors have already studied the relationship between g_s and k_L , concluding that in most species, including grapevine, both conductances are tightly correlated (17, 18, 21, 29, 39). While aquaporins, in roots, act as the entrance valves for water (20, 28), stomata

in leaves act as water vapour exit valves that limit transpiration (E).

However, recent insights, cast doubt on the main role of stomatal closure on the embolism-avoidance strategy (38, 39), besides the fact that the actual involved mechanism is still not elucidated (2, 16).

In addition, grapevines have shown to own a highly resistant xylem (10) that cavitates at higher tensions than previously thought, keeping k_L between certain values before stomata respond. In this context, this research intended to study the cavitation phenomenon in grapevines and the mechanisms involved in its control. It tried to comprehend on a mechanistic manner, the stomatal functioning, its relation to the cavitation phenomenon, and the physical laws that rule them. This was achieved by complementing the construction of a functional and dynamic model with the comparison of two contrasting varieties, Syrah and Grenache, under two different water treatments, grown in pots, inside a greenhouse. These varieties were chosen because they have been reported as opposite in regards to stomatal behaviour, isohydric and anisohydric, respectively (8, 9), However, this classification is currently under strong debate (8, 14, 19, 21, 29). Given this controversy, tried to try the model as well as the varieties' behaviour under these conditions.

Model developing

Several models have been developed describing and explaining the stomatal functioning (8). The model includes several sub-models and relates them in an attempt to explain embolism control by hydraulic traits in grapevine, adding the "vulnerability to cavitation element", and clarifying the coupling mechanism that achieves embolism control.

This model is based on the Ohm's law analogue concept (37) that states that the flow (J_w) escaping through stomata, called transpiration (E), constitutes the impulsive force that drives water along the xylem vessels. This suction that occurs inside the xylem vessels is expressed in terms of water potential (Ψ ; MPa). Finally, this Ψ difference between soil and leaves ($\Delta\Psi$) is what allows water to move from one place to the other (13, 37).

$$J_w = \frac{\Delta\Psi}{R} = k_L \cdot \Delta\Psi = E; \quad (1)$$

$$\Delta\Psi = \Psi_{soil} - \Psi_L \quad (2)$$

where:

$J_w = E$, is transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)
 R = hydraulic resistance = $1/k_H$ ($1/(\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \text{MPa}^{-1})$)
 k_L = hydraulic conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$)
 $\Delta\Psi$ = water potential difference (MPa)
 Ψ_{soil} = soil water potential (MPa)
 Ψ_L = leaf water potential (MPa)

Assuming that species like grapevines have null capacitance (25); (meaning that there is no water storage due to the water potential difference), J_w equals E (1), and may be expressed by Fick's law as follows:

$$E = \frac{(e_{sT(L)} - e_a)}{P_a \cdot (g_s^{-1} + g_b^{-1})} \quad (3)$$

where:

E = transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)
 $(e_{sT(L)} - e_a)/P_a = q'$ which is the difference of water vapour concentration between leaf and atmosphere, the vapour pressure gradient from leaf to air (dimensionless variable, it is a ratio of pressures).
 $(g_s^{-1} + g_b^{-1})$ = sum of stomatal and boundary layer resistances ($1/\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$).

Then, by replacing (2) and (3) in (1), is obtained (4)

$$\Psi_L = \Psi_{soil} - \frac{q' / g_s^{-1} + g_s^{-1}}{k_L} \quad (4)$$

where:

q' = vapour pressure gradient from leaf to air.

Eq. (4) formalizes the relationship between Ψ_L , $q'g_s$ and k_L .

The next step in this model development is to relate plant embolism (Emb) to the hydraulics described. Emb inversely depends on water potential (Ψ). For more negative values of Ψ , higher percentages of Emb can be measured. In the model, Emb was interpreted by means of the mathematical adjustment of the sigmoid vulnerability curves of grapevines to a piece-wise defined function, shown as follows in figure 1.

In the first piece of the function, up to certain $\Psi_L = \Psi_{L1}$, Emb equals zero (5). This part of the function is called "lag" and corresponds to a range of Ψ_L values where no embolism takes place. When Ψ_L diminishes because of increasing water deficit, exceeding Ψ_{L1} , Emb linearly depends on Ψ_L , and grows until maximum Emb -i.e. 100%- is achieved for $\Psi_L = \Psi_{L2}$ (6). On or after Ψ_{L2} , Emb equals 1 (or 100%; (7); figure 1).

$$\begin{cases} Emb = 0 & ; \Psi_L \leq \Psi_{L1} \end{cases} \quad (5)$$

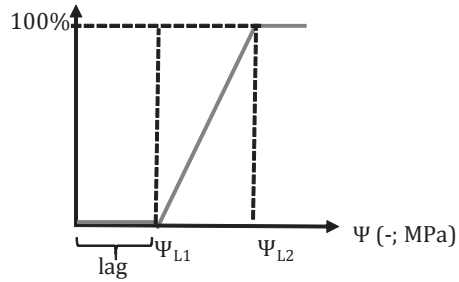
$$\begin{cases} Emb = a + b \cdot \Psi_L; \Psi_{L1} \geq \Psi_L \geq \Psi_{L2} \end{cases} \quad (6)$$

$$\begin{cases} Emb = 1 & ; \Psi_L \leq \Psi_{L2} \end{cases} \quad (7)$$

By trigonometry, equation (6) can mathematically be expressed as (8):

$$Emb = \frac{\Psi_L - \Psi_{L1}}{\Psi_{L2} - \Psi_{L1}}; \Psi_{L1} \leq \Psi_L \leq \Psi_{L2} \quad (8)$$

Then, by replacing (4) in (6) is obtained the suffered Emb as output of the model, (Equation 9), for the part of the function in which Emb linearly depends on Ψ_L .



The "lag" indicates the pressures under Ψ_{L1} where no embolism takes place. Note that Ψ are negative values.

El "lag" indica la presión bajo la cual no existe embolia. Los valores de Ψ son negativos.

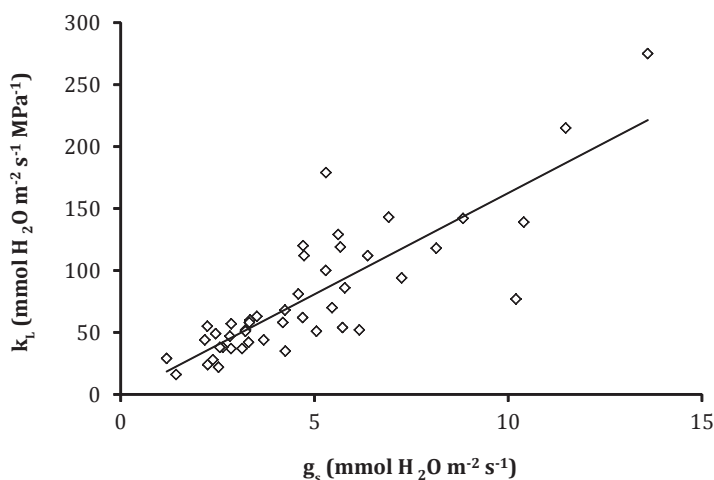
Figure 1: Theoretical vulnerability curve for grapevines. Ψ_{L1} , the pressure at which embolism starts, and Ψ_{L2} , the pressure at which embolism reaches its maximum value.

Figura 1: Curva teórica de vulnerabilidad para vid. Ψ_{L1} , la presión a la cual comienza la embolia, y Ψ_{L2} , la presión a la cual existe embolia máxima.

$$Emb = \frac{(\Psi_{soil} - \frac{q' / g_s^{-1} + g_b^{-1}}{k_L}) - \Psi_{L1}}{\Psi_{L2} - \Psi_{L1}} \quad (9)$$

Equation 9 probes that plant embolism effectively depends on g_s , although not in a direct manner, but through its interaction with other variables, like q' (that depends on leaf temperature T_L and atmospheric variables), the boundary layer conductance (g_b , that varies with wind speed) and most importantly, with k_L , that depends on root aquaporin activity (36). With respect to the g_s vs. k_L relation, it has been widely proved that they are strongly correlated throughout the day; and this study corroborated this fact ($R = 0.8438$, $p = 0.000$, figure 2, page 37).

In this study, it is created a new variable called Δg_s that represents the ratio between g_s and k_L .



Values are individual measurements. / Los puntos son valores individuales de medición.

Figure 2. Correlation between stomatal conductance (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) and hydraulic conductance (k_L , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) for Syrah.

Figura 2. Correlación entre conductancia estomática (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) y conductancia hidráulica (k_L , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) para Syrah.

The model also shows the feedback relationship between Emb and k_L . This feedback process states that Emb depends on k_L , while, at the same time, k_L depends on Emb, as the former diminishes when the last rises.

Figure 3 (page 38), shows the dynamic mechanistic model that explains how the relationship between g_s and k_L controls embolism, and how embolism, in turn, modifies k_L in a feedback loop. The k_L (2) is the result of a k_L before embolism (k_{Lbe} or maximum k_L , 3), then affected by embolism (1). The k_{Lbe} is a function of the time of day and Ψ_{soil} .

The model adopted several already existing models to fit into the general model.

The g_s (5) is interpreted by the Buckley *et al.* model (2003); g_b and leaf temperature (T_L) are expressed by the Campbell and Norman (2012) equations (4); Ψ_{ng} (osmotic water potential of the guard cell),

included in g_s (5), is empirically expressed by Taiz and Zeiger (1998) and Tardieu and Simonneau (1998). The entry variables are Ψ_{L1} , Ψ_{L2} , time of day (hour), Ψ_{soil} , T_a , e_a/P_a , wind speed and solar radiation. The model output is Embolism (1).

Model parameterizing

To parameterize the model, it was designed an experiment with Grenache and Syrah plants, under field capacity and water stress. It was measured gas exchange and Ψ_L during a complete day, from predawn to 18 h. The k_L was calculated for each moment along the day, and a time dependent equation was then fitted to the data. Vulnerability curves were constructed and embolism achieved along the day was estimated.

$$\Psi_{L1}, \Psi_{L2}, \text{time(hour)}, \Psi_{\text{soil}}, T_a, q', \text{wind, solar radiation; entry variables} \quad (1)$$

$$\begin{cases} Emb = 0 & ; \Psi_L \leq \\ Emb = a + b \cdot \Psi_L & ; \Psi_{L1} \geq \Psi_L \leq \Psi_{L2} \\ Emb = 1 & ; \Psi_L \leq \Psi_{L2} \end{cases} \quad (2)$$

$$k_L = k_{Lbc} (1 - Emb) \quad (3)$$

$$k_{Lbc} = f(\text{time}, \Psi_{\text{soil}}) \quad (4)$$

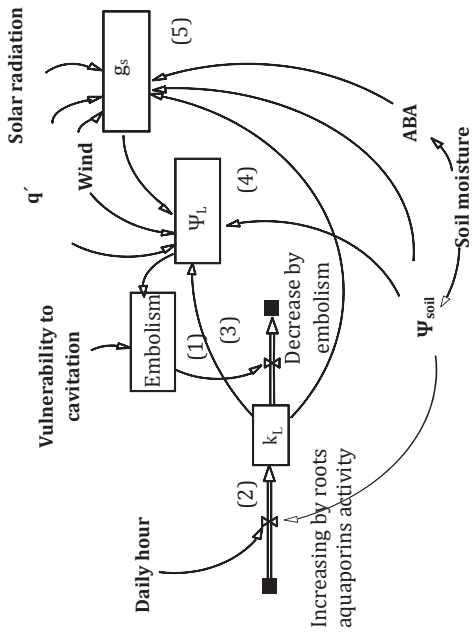
$$\Psi_L = \Psi_{\text{soil}} \frac{q' / g_s^{-1} + g_b^{-1}}{k_L} \quad (5)$$

$$g_b = 1.4 \cdot 0.147 \sqrt{\frac{\mu}{0.8} w_L} \quad \text{Campbell and Norman (2012)}$$

$$q' = (es(TL) - ea) / Pa \quad \text{Taiz and Ziegeer (1998)}$$

$$\Psi_{ng} = f(\text{solar radiation}, [ABA]) \quad \text{Tardieu and Simmoneau (1998)}$$

$$g_s = \frac{x \cdot (-\Psi_{ng} + \Psi_{\text{soil}} - M \cdot \Psi_{\text{soil}} + M \Psi_{\text{ng}})}{1 - X \cdot (M \cdot k_L^{-1} - f_g \cdot r_g \cdot P)} \quad \text{Buckley et al. (2005)}$$



Equations on the right are represented by numbers in the figure. The ambient mechanics are formalized by the Campbell and Norman (1998) equations of boundary layer conductance (g_b) and atmospheric deficit (q') that are then included in the Buckley et al. (2005) equation for stomatal conductance (g_s) (5). The Van den Honert (1948) equation (4) establishes the leaf water potential (Ψ_L) that later will define plant hydraulic conductance (k_L). The hydraulic conductance before embolism (k_{Lbc}) equation formalizes the relationship between maximum k_L and k_L after embolism takes place, being the formal subjected to soil water potential and daytime, according to Vandeleur et al. (2009) (2) and (3). Finally, the equations expressing Embolism course are shown in (1). Equation (1) defines Xylem Embolism based on the parameters that characterize the vulnerability curve of a definite species. Note that this model is constructed based on several mechanistic models, and all of them joined together in one bigger mechanistic model via the Embolism functional equation.

Las ecuaciones de la derecha se representan por números en el diagrama. Los mecanismos ambientales se formalizan mediante las ecuaciones de Campbell y Norman (1998) para la conductancia de la capa límite (g_b) y el déficit atmosférico (q') que luego se incluyen en la ecuación de Buckley et al. (2005) para conductancia estomática (g_s) (5). La ecuación de Van den Honert (1948) (4) define el potencial hídrico foliar (Ψ_L) que luego definirá la conductancia hidráulica (k_L). La ecuación de conductancia hidráulica antes de la embolia (k_{Lbc}) formaliza la relación entre la k_L máxima y la k_L después de la embolia. La primera depende del potencial hídrico del suelo (Ψ_{soil}) y el momento del día, según Vandeleur et al. (2009) (2) and (3). Finalmente, las ecuaciones que expresan el curso de la Embolia se muestran en (1). La ecuación (1) define Xylem Embolism basándose en los parámetros que caracterizan la curva de vulnerabilidad de una especie definida. Nótese que este modelo integra modelos mecanísticos previos en uno solo, mediante la ecuación de embolia de la vid.

Figure 3. The dynamic model was formulated to explain how embolism control takes place by stomatal and hydraulic coupling.

Figura 3. El modelo dinámico se construyó para explicar cómo la embolia es controlada por el acople entre la conductancia estomática e hidráulica.

MATERIALS AND METHODS

Vines and site

The experiment was undertaken during the season 2012/2013 at the INTA's Experimental Station, in Mendoza, Argentina.

A factorial experiment combining 2-year-old Syrah and Grenache grapevines and two water regimes was established on the summer of 2012. In quadruplicate, dormant own-rooted vines were removed from their 4-L pots and replanted on a sandy loam substrate on 15-L pots to allow good growth during the season.

Water regimes, named field capacity (FC) and water deficit (WD), were irrigated with 100% and 50% of the fraction of transpirable soil water (FTSW), respectively, as follow. Immediately after replanting all vines were irrigated to saturation and water treatments were applied; the FC treatment was watered every two days to maintain 100% FTSW whereas the WD treatment was left without irrigation for a week until it reached the targeted soil moisture of 50% FTSW (0.16 g/g). After the WD pots achieved the desired soil moisture, pots were watered every two days replenishing the transpired water. Water regimes were maintained for three months. Moisture was measured every two days using moisture probes (ECH20 EC-5 sensors, Decagon devices, USA). Vines were trained to one shoot and grown in a greenhouse with daily average temperature of 25°C and photosynthetic active radiation of 800 $\mu\text{moles m}^{-2} \cdot \text{s}^{-1}$.

Water potential and gas exchange measurements

A portable photosynthesis system (CIRAS-2, PP Systems, Hertfordshire U.K) was used to measure instantaneous leaf gas exchange. Measurements were carried out every two hours from 6am and 6pm.

The CO_2 concentration of the incoming air was maintained at 375 $\mu\text{mol} \cdot \text{mol}^{-1}$. The same leaves used for gas exchange assessment were used to measure leaf water potential (Ψ_L), with the Scholander pressure chamber (Biocontrol, Córdoba, Argentina), using the procedure of Hsiao (37). Predawn water potential (Ψ_{PD}) was considered as a proxy to soil water potential (Ψ_{soil}).

Water vapour concentration at leaf temperature ($e_{sT(L)}$ /Pa; hPa), was calculated via the equation of Tetten.

$$e_{sT(L)} = 6.11 \times \text{Exp} \left(\frac{17.502 \times T_L}{T_L + 240.97} \right) \quad (10)$$

where:

T_L = leaf temperature

Hydraulic conductance was calculated through the Van den Honert law, with Ψ_{soil} , Ψ_L , and E for every assessed moment of the day.

Leaf embolism along the day (Emb) was estimated from the daily course curves of Ψ_L and the vulnerability curves, for each plant. Both curves were related and the positive pressures achieved for the vulnerability curves were directly linked to the Ψ_L measured along the day, assigning an embolism value to each moment and plant.

Vulnerability curves

The loss of k_H to increasing Ψ_L , i.e. cavitation, was studied by constructing vulnerability curves for each plant: after all water potential and gas exchange measurements finished, shoots were harvested and transported to the laboratory for vulnerability curves construction. Previously, every leaf was removed from the stem, and the cut surfaces were sealed with contact glue. Vulnerability

curves were constructed following the "Air Injection Long" method already described (10), using a double ended pressure sleeve connected to a Scholander pressure chamber (Biocontrol, Córdoba, Argentina;). First, the shoots were flushed for 30 minutes using distilled, degassed 5% potassium hypochlorite (KClO) solution, removing all embolisms and obtaining maximum k_H ($k_{H_{max}}$). Then, successive pressure cycles were imposed. The air pressure in the chamber was increased to a specific value and held for 10 minutes before it was reduced back to zero. Air pressure was successively increased to higher levels and hydraulic measures were taken, obtaining the k_H for each cycle. Percentage loss of conductivity (PLC) was calculated for each cycle relative to $k_{H_{max}}$.

$$PLC = 100 \times (1 - (k_i / k_{max})) = (k_{max} - k_i) / k_{max}$$

Statistics and data analysis

Differences between treatments were assessed by multifactor and one-way ANOVA, followed by LSD test ($p < 0.05$) using Stat Graphics Plus (Statistical Graphics Corp.; StatSoft, Inc., 2003). When homogeneity of variance was not reached, non-parametrical analyses were carried out.

RESULTS

Water relations: water potential and stomatal conductance

The imposed water deficit had an evident effect on Ψ_L . The water deficit (WD) treatments provoked a significantly lower (more negative) Ψ_L than those of the field capacity (FC) plants. For midday water potential (Ψ_{md}), interaction between treatments was significant (varieties vs. water treatments; $p = 0.0333$). As for stomatal conductance (g_s), the WD treatments had significantly lower values than the FC ones, for the whole day course ($p = 0,01$; figure 4, page 41).

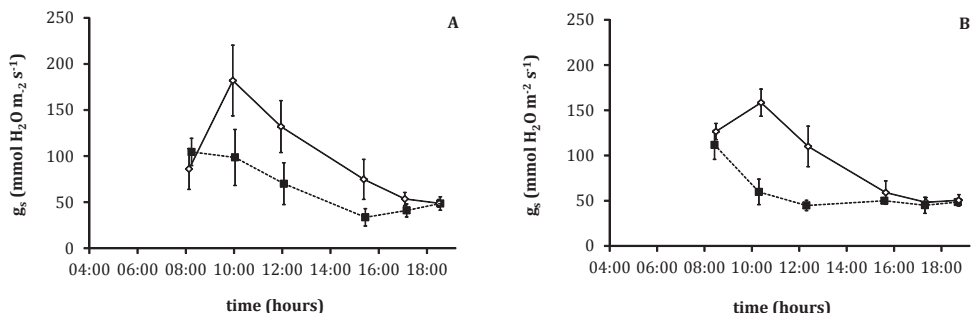
Once all vulnerability curves were obtained, each one was adjusted to a piecewise defined function as described in the "Model developing" section: The first piece of the function, where no cavitation or embolism (Emb) has already happened, is called "lag" and equals zero for Emb; while the second part of the function, showing increasing cavitation, is fitted to a straight line equation (figure 1, page 36 and figure 5, page 41).

The ANOVA analysis for the straight line fitting parameters a (intercept) and b (slope) showed that neither statistically significant interaction, nor significant differences existed among varieties or water treatments (table 1, page 42). This means that under the experimental conditions, no xylem adaptation upon water stress occurred.

Xylem embolism vs. water relations, throughout day

Estimated embolism throughout the day was only achieved in four cases, because most of the plants had vulnerability curves with long lags that started from -1.5 MPa, while this Ψ_L value was generally not achieved in the greenhouse. As leaf embolism directly depends on Ψ_L , both variables followed similar, though opposite daily courses. Lower Ψ_L corresponded to higher cavitation values (figure 6, page 42).

When it was observed the daily courses of embolism and g_s for the four plants that did cavitate, it was observed that there was no relation between both variables that could explain embolism control. Stomatal closure events (reduction on g_s) vs. embolism detention did not correlate throughout the day, meaning that stomatal conductance, *per se*, is independent of embolism (figure 7 page 42). This conclusion was already achieved theoretically by means of the model that clearly showed that the coupling between g_s and k_L (and not g_s alone), is, in fact, the controlling switch.

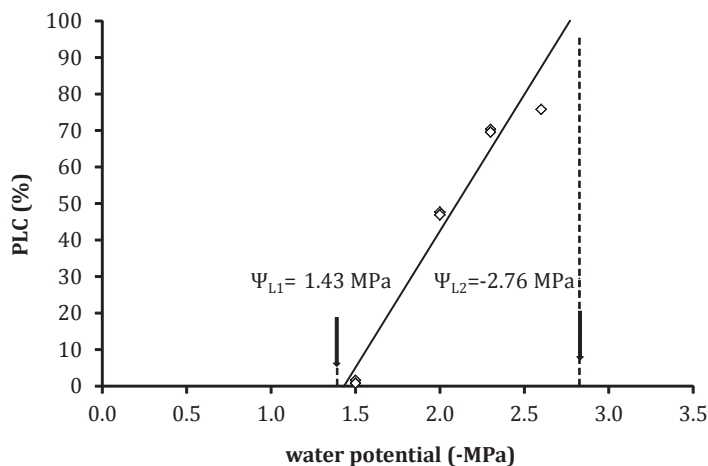


Each point corresponds to the mean \pm one SE for water deficit (WD; dotted line) and field capacity (FC; solid line) treatments.

Cada punto corresponde a la media \pm un error para déficit hídrico (WD; línea de puntos) y capacidad de campo (FC; línea entera).

Figure 4. Daily course of stomatal conductance (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) for Grenache (A) and Syrah (B).

Figura 4. Dinámica de la conductancia estomática a lo largo de un día (g_s , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) para Grenache (A) y Syrah (B).



Dots indicate values from a single shoot. Straight line indicates fitted linear curve.

Los puntos indican los valores medidos en un tallo. La línea indica el ajuste lineal obtenido.

Figure 5. Vulnerability curve measured in Grenache under water stress [percentage loss of hydraulic conductance (PLC) vs. pressure (Ψ_L)]. The pointed Ψ_{L1} and Ψ_{L2} are the pressures at which xylem embolism starts and equals 100%, respectively. Under Ψ_{L1} no embolism takes place.

Figura 5. Curva de vulnerabilidad medida en Grenache bajo estrés hídrico [porcentaje de pérdida de conductancia hidráulica (PLC) vs. presión (Ψ_L)]. Los valores de Ψ_{L1} y Ψ_{L2} señalados son las presiones a las que la embolia comenzó y alcanzó el 100%, respectivamente. Debajo de Ψ_{L1} no existe embolia.

Table 1. Straight line fitting parameters b (slope) and a (intercept) for vulnerability curves in Grenache and Syrah cultivars, under field capacity (FC) and water deficit (WD). Data are means and p -values are from the ANOVA.

Tabla 1. Parámetros de ajuste lineal b (pendiente) y a (intercepto) para curvas de vulnerabilidad en Grenache y Syrah, bajo capacidad de campo (FC) y déficit hídrico (WD). Los datos son medias de mediciones y los p -values provienen del ANOVA.

Variable	b	a
Cultivar (C)		
Grenache	-0.8855	-1.9221
Syrah	-0.8678	-1.4316
p value	0.9615	0.6209
Water treatment (W)		
FC	-0.6077	-1.0930
WD	-1.1457	-2.2607
p value	0.1596	0.2501
p value (C \times W)	0.8775	0.9983

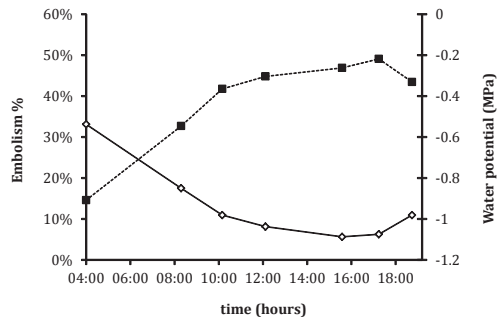


Figure 6. Daily course of water potential (Ψ_L ; solid line) and embolism (dotted line). Values are means from the three Syrah vines and the one Grenache plant suffering embolisms throughout the day.

Figura 6. Dinámica del potencial hídrico (Ψ_L ; línea entera) y la embolia (línea punteada) a lo largo del día. Los valores son la media para tres plantas de Syrah y una planta de Grenache en condiciones de embolia.

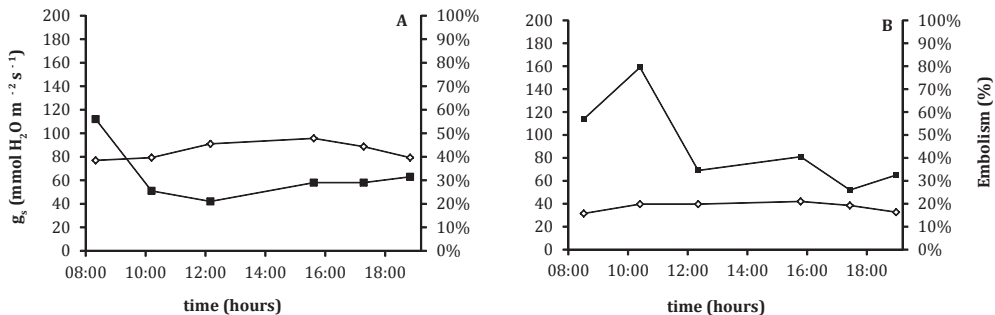


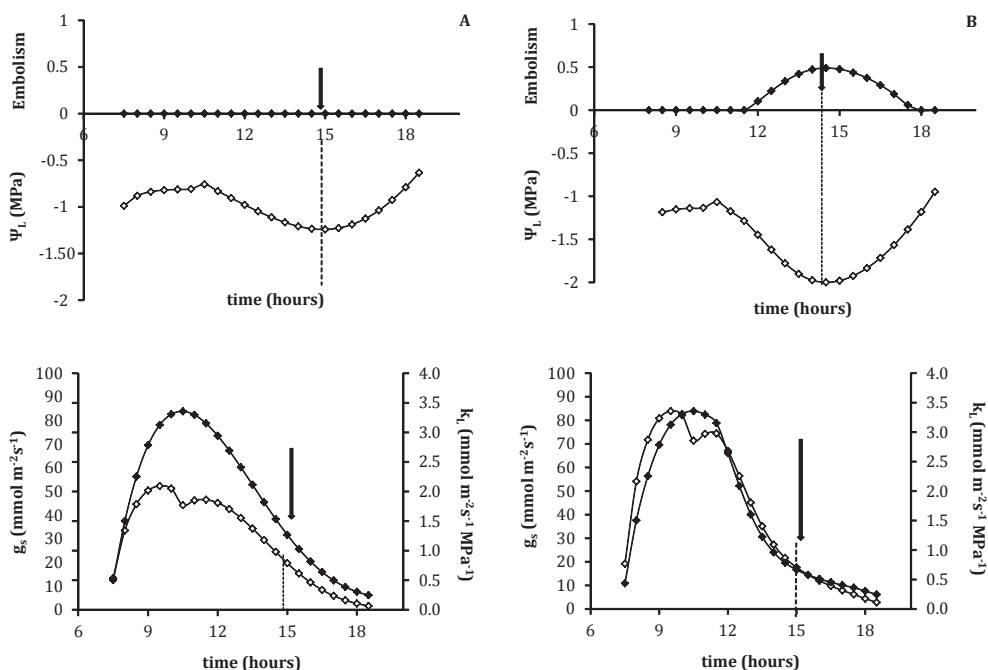
Figure 7. Daily course of stomatal conductance (g_s ; black squares) and embolism (black diamonds) for two vines that suffered embolism under no stress (A) and under water deficit (B). Clear independence between both curves is shown.

Figura 7. Cinética de la conductancia estomática (g_s ; cuadros negros) y la embolia (diamantes blancos) a lo largo de un día para dos vides que sufren embolia bajo condiciones de riego suficiente (A) y bajo estrés hídrico (B). Notar la clara independencia entre ambas curvas.

Simulations

Figure 8, shows how embolism, Ψ_L , g_s and k_L behaved under water stress ($\Psi_{soil}=-0.2$), constant ambient circumstances and a variable guard cell osmotic adjustment (π_g). The π_g modifies, in a significant manner, the stomatal adjustment (Buckley *et al.*, 2003). In this case, it was modified π_g by increasing its value by 20% for case A; and diminishing

π_g by 20% in case B. Notice that as Ψ_L gets more negative and achieves -2 MPa, embolism starts and g_s couples with k_L ($\Delta g_s = 28$), allowing a maximum embolism of 45% for simulation B. For simulation A, where Ψ_L does not grow over the threshold value and no embolism takes place, the coupling is much less evident ($\Delta g_s = 16$).



Arrows in figure A show that no embolism occurs, stomatal conductance (g_s) does not couple with hydraulic conductance (k_L) and $\Delta g_s=16$. Arrows in figure B show that as soon as embolism starts, stomatal conductance (g_s) couples with hydraulic conductance (k_L) achieving a $\Delta g_s=28$. (Embolism and k_L ; black diamonds; Ψ_L and g_s ; white diamonds).

Las flechas en la figura A muestran que la embolia no ocurre, la conductancia estomática (g_s) no se acopla con la conductancia hidráulica (k_L) y $\Delta g_s=16$. Las flechas en la figura B indican que en cuanto comienza a haber embolia, la g_s se acopla con la k_L alcanzando un $\Delta g_s=28$. (Embolia y k_L ; rombos negros; Ψ_L y g_s ; rombos blancos).

Figure 8. Simulations of embolism, Ψ_L , g_s and k_L under water stress ($\Psi_{soil}=-0.2$), constant ambient circumstances and variable guard cell osmotic adjustment (π_g).

A: π_g augmented by 20%. B: π_g diminished by 20%.

Figura 8. Simulaciones de embolia, Ψ_L , g_s y k_L bajo estrés hídrico ($\Psi_{soil}=-0.2$), circunstancias ambientales constantes y ajuste osmótico de la célula guardiana variable (π_g). A: π_g aumentado un 20%. B: π_g disminuido un 20%.

DISCUSSION

These measurements and the ideated mechanistic model, demonstrated that embolism and g_s are independent. Embolism depends on g_s in addition to other physiological and ambient variables; like xylem vulnerability, k_L , difference on water vapour between leaf and atmosphere, and boundary layer conductance. In this study g_s and k_L were tightly associated ($R = 0.70$). Through the model, it could be shown that the daily embolism restraint was linked to the variation that g_s suffered in intimate relation with k_L , and not to g_s itself. This tight relation between both conductances has already been widely observed in grapevine and trees (17, 21, 29, 39). This puts in evidence that g_s responds to variations in k_L ; and that both variables, in mutual interaction, control embolism in grapevines.

Apparently, k_L and g_s are related because, under drought, stomata operate allowing photosynthesis and preventing desiccation at the same time (7, 8). Consequently, g_s must respond to k_L , since changes in k_L influence plant and leaf water status (17). Therefore, the effect of g_s as prime embolism restraint attributed in grapevines and other species, could be related to the fact that both conductances are strongly coordinated (21, 29, 39).

In relation to the generated model, it should be settled that the input variable k_L is affected by the intrinsic embolism level, including a feedback process.

The model measures the phenomenon while k_L grows, (and it grows despite of the portion of hydraulic conductivity that embolism captures, probably by the action of the root aquaporins) (36).

In fact, the model functions calculating embolism in a time t , from embolism in time

$t-1$ (integrated in the input variable, k_L). This is incorrect in negative feedback mechanisms, as embolism control shows to be (30). It might also be probable that this embolism that affects k_L is, partly, responsible for the stricter coupling of g_s and k_L . Nardini and Salleo (2000), explained that in many species embolism cannot be completely avoided and that it could constitute the signal that stomata need to start closing up. This can be reinterpreted as follows: stomata actually respond to a lower k_L caused by certain embolism formation, coupling itself to this changing k_L .

The obtained vulnerability curves were not different among treatments. Therefore, for the achieved water deficit, no xylem adaptation took place. It might be possible that differences among varieties were not evidenced because the stress levels achieved were not severe enough to generate these adaptation responses. It could also be possible that the three months period during which the plants lived was not long enough to let the xylem system anatomically adapt to the stressful situation. Besides, the possibility of discriminating vulnerability differences turns to be quite hard, given that the phenomenon shows great intrinsic variability in grapevines as in other species, (21, 22, 23, 35). For this study, variation coefficient for the "lag" value was 0.55.

Of great importance is to highlight that in grapevines, embolism control does not require complete stomatal closure, meaning that photosynthesis is not completely deprived, and the assimilation cost is not as high as expected for other species.

In this experiment, under severe water stress ($\Psi_{\text{soil}} = -0.2$ MPa, and 50% embolism) g_s was significantly reduced but never achieved complete stomatal closure. In less stressful conditions, embolism is well controlled while stomata are maintained opened.

In 2011, Zufferey *et al.* (2011) found that stomata closed up only after 90% of embolism was achieved. This means that

the plant can keep on photosynthesizing and, at the same time, avoid catastrophic cavitation (cavitation levels at which the plant cannot recover and dyes). In this sense, it is one remarkable species that may require low amounts of water, and still produce quantity and quality of fruit, without the risk of suffering severe embolism events.

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Planting density and its effect on plant height and rosette quality in ornamental cabbages for cutting

Efecto de la densidad de plantación sobre altura de planta y calidad de roseta en repollos ornamentales de corte

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ABSTRACT

Brassica oleracea L. var. *acephala* is an ornamental cabbage cultivated as foliage for cutting due to the coloration of the rosette formed by its apical leaves. The plant height and the diameter of the rosettes may be modified through the planting density; however, there is not enough information about this. The aim of this study was to assess the influence of the planting density of four cultivars of *B. oleracea* L. var. *acephala* of the series F1 Crane: Bicolor, Pink, Red and Rose, on the plant height, rosette diameter, number of leaves, leaf area, root volume, and aerial and root fresh and dry weight. The experimental design was completely randomized, with six repetitions. The combination of density (56 plants.m⁻² and 28 plants.m⁻²) and cultivar allowed obtaining eight treatments. The results showed that the treatments with high planting density improved commercial quality indices: achieving greater stem height and more compact and smaller diameter rosettes, leading to lower values of the rest of the vegetative variables evaluated. Also, as productive alternative, it would allow a more efficient use of the soil and reduce crop management activities such as manual leaves removal.

Keywords

Brassica oleracea L. • foliage for cutting • market quality

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RESUMEN

Brassica oleracea L. var. *acephala* se cultiva como follaje para corte debido a la coloración de la roseta de hojas apicales de este repollo ornamental. Es posible modificar la altura de las plantas y el diámetro de las rosetas, para mejorar la calidad comercial, a través de la densidad de plantación, sin embargo, no hay suficiente información al respecto. Se evaluó la influencia de la densidad de plantación en cuatro cultivares de la serie F1 Crane: Bicolor, Pink, Red y Rose, sobre altura del tallo, diámetro de roseta, número de hojas, área foliar, volumen radical y peso fresco y seco, particionados en vástago y raíz. El diseño experimental fue completamente aleatorizado (DCA), con seis repeticiones. Por combinación de factores densidad (56 pl.m^{-2} y 28 pl.m^{-2}) y cultivar se obtuvieron 8 tratamientos. Una alta densidad de plantación aumentó la calidad comercial de las plantas, las que tuvieron rosetas apicales más compactas y de menor diámetro y mayor altura del tallo, reduciendo el resto de las variables vegetativas. Asimismo, permitiría un uso más eficiente de la tierra disminuyendo tareas de manejo del cultivo tales como el deshoje de las varas.

Palabras clave

Brassica oleracea L. • follaje para corte • calidad comercial

INTRODUCTION

Brassica oleracea L. var. *acephala* is a biennial plant usually grown as an annual for ornamental purposes. Ornamental cabbages for cutting are used as substitutes for flowers during autumn and winter due to the decorative aspect of their central leaves, that display different colors, which are enhanced by the degradation of chlorophylls induced by low temperatures (13). Unlike that observed in other varieties, the central leaves of *B. oleracea* L. var. *acephala* develop a rosette-like shape, without forming a 'head' at the top of the plant (11). At the time of harvest, the lower leaves are removed, leaving the rosette on the top of the plant to give it a flower-like appearance.

The commercial production of ornamental cabbages usually takes place by the end of the summer, when warm temperatures promote the growth of plants. Growth stops when night

temperatures are below 15°C , time at which the central and upper leaves express their characteristic coloration. An important management tool for the commercial production of ornamental cabbages is the choice of the number of plants per unit area because several factors such as the absorption of light, the consumption of water and nutrients, and the possible incidence of diseases depend on this variable.

According to different researchers, in order to satisfy the needs of the market, the size and composition of edible broccoli may be modified by different fertilizer (26) but ornamental cabbages for cutting may be modified through the management of the planting density. However, there is still not enough information on this issue. Thus, the aim of the present study was to assess the differences in plant height and rosette diameter, which are ornamental quality

indexes, as well as in other vegetative variables in four cultivars of the series F1 Crane of *B. oleracea* L. var. *acephala* under two planting densities. A higher density of plants leads to cabbages of greater height and smaller rosette diameter, and reduces the individual growth of cabbages, affecting other components such as leaf area, number of leaves, root volume, and aerial and root fresh and dry weight.

MATERIALS AND METHODS

The experiment was carried out in the experimental field of the Cátedra de Floricultura of the Faculty of Agriculture of Buenos Aires University (34°40' S, 58°30' W). Four cultivars of *B. oleracea* L. var. *acephala* of the series F1 Crane (American Takii Inc.) were used: Bicolor, Red, Rose and Pink. Cultivation began on February 3rd, 2012, starting from seedlings from the propagation company, on black plastic plug trays (150 cells of 28 cm³ each), with a substrate mix of 50:50 peat and perlite. The experiment was carried out in a greenhouse under controlled conditions for 14 days, time at which plants were transplanted to the field and sown in 0.8 m x 6.3 m flowerbeds raised 0.2 m above the ground.

For each cultivar, two treatments with different planting densities were carried out: high density (56 plants m⁻²) and low density (28 plants m⁻²), with a planting diagram of 12.5 x 15.0 cm and 25 x 30 cm respectively. It was used a tutoring mesh (grid of 15 cm x 12.5 cm) that was raised as plants grew. Drip irrigation was managed according to the needs of the crop, and the necessary phytosanitary controls were carried out. Harvest took place when the upper leaves formed a colored center, characteristic of each cultivar. Data of

the mean daily temperature during the experimental period were obtained from the Central Observatory of Buenos Aires (34°35'24"S 58°29'0" W).

The crop variables evaluated were: i) rosette diameter, determined at harvest as the average value of two perpendicular measures; ii) plant height, recorded on a weekly basis throughout the study with a metric tape from the soil surface up to the apex of the stem; iii) leaf area per plant, determined at harvest time and estimated by separating and scanning the leaves of each plant; the images were analyzed using ImageJ software; iv) number of leaves per plant, counted twice a week throughout the study, taking as a criterion the fully expanded leaf; v) root volume per plant, determined at harvest time by dipping the root (separated from the stem) in a test tube with a known volume of water (100 mL) and subsequently measuring the volume of the displaced water; vi) rate of leaf appearance, calculated from the cumulative number of new leaves per plant as a function of the elapsed time; vii) aerial fresh weight (AFW), considering the stem and the "rosette" of leaves, composed of two cycles of green leaves (eight leaves) taken from the colored center; viii) root fresh weight (RFW), determined by cutting the roots taking as a point of reference the first node located above their insertion; and ix) aerial and root dry weight (ADW and RDW respectively), determined by separating the stems and the leaves from the root and placing them in a stove at 70°C until consistency of weight. A TH-200 digital scale (accuracy 0.1 g) was used for all weight measurements. The measurements of all the studied variables were completed on April 23rd, 2012.

The design was fully randomized with a 2 x 4 (density and cultivar) factorial

arrangement, with the plant as experimental unit and six repetitions per treatment. The statistical analysis was performed using ANOVA and Tukey's Test ($p = 0.05$), using the Infostat software InfoStat / Professional V1.1 (8).

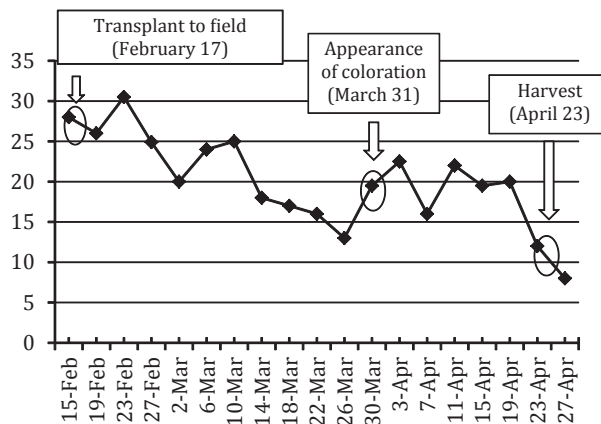
RESULTS

The evolution of the mean temperature during the experimental period presented a slightly descending trend, according to the change of season (summer-autumn). Coloration in the central leaves began to appear once mean temperatures showed a significant decrease (figure 1). This change was verified simultaneously in all the cultivars and showed to be gradually intensified in the subsequent days.

Regarding the plant height, the curves of the eight treatments showed a similar pattern of response, but with different magnitudes, which began to be obvious several weeks after planting.

The *cv. Bicolor* showed the greatest increases in plant height, mainly under the condition of high density, and, in the case of low density, in a shorter interval (with significant differences ($p \leq 0.05$) respect to the *cv. Pink* and *Rose*). At the end of the experiment, the plants cultivated at high density showed significant differences with respect to those planted at low density in all cultivars, except in *Pink* (figure 2, page 53).

All together, the plants grown at high density presented a final mean height that was 18.4% higher than that of plants grown at low density, with significant differences between them.



The arrows show the moments of the field transplant, appearance of coloration in central leaves and harvest. Las flechas indican los momentos de transplante a campo, aparición de color en hojas centrales y cosecha.

Figure 1. Evolution of the mean temperature (°C) during the crop cycle (summer-autumn 2012).

Figura 1. Evolución de la temperatura media durante el ciclo del cultivo (verano-otoño 2012).

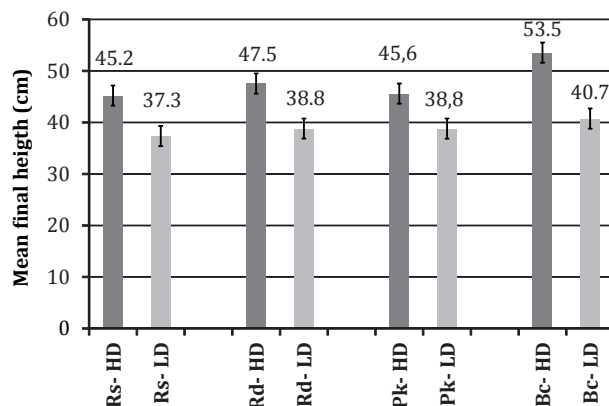


Figure 2. Mean final height (cm) in ornamental cabbage plants (*Brassica oleracea* L. var. *acephala*) cv. Rose (Rs), Red (Rd), Pink (Pk) and Bicolor (Bc) at high planting density (HD) (56 plants/m²) and low planting density (LD) (28 plants/m²).

The final height is the mean value \pm standard error.

Figura 2. Altura final promedio (cm) (valor medio \pm error estándar) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) de los cv. Rose (Rs), Red (Rd), Pink (Pk) y Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/m² y baja densidad (BD) 28 plantas/m².

A significant density x cultivar interaction for plant height was found only for the first fifteen days from planting, whereas in the successive periods, the effects of both variables were independent (table 1, page 54).

Rosette diameter was significantly affected by planting density and cultivar, but not by the interaction between these factors. This variable was smaller in plants sown at high density than in those sown at low density, with significant differences in all cultivars (figure 3, page 54).

The mean rosette diameter of all the plants grown at high density was 32.7% smaller than that of those grown at low

density, with significant differences ($p \leq 0.05$).

On the other hand, without considering the effect of density, it was found differences between cultivars, where the greatest mean rosette diameter was that of Bicolor, which was significantly different from that of Pink ($p \leq 0.05$). Both the planting density and the cultivar showed a significant effect on this variable, whereas the interaction of both factors did not.

The leaf area per plant presented significant differences between densities as well as between cultivars, but no density x cultivar interactions were found.

Table 1. Mean final height (cm) of the plant as a function of the planting density and cultivar in *B. oleracea* L. var. *acephala*.

Tabla 1. Valores medios de altura final (cm) de la planta en función de los factores densidad y cultivar en repollos de *B. oleracea* L. var. *acephala*.

Density	Final height (cm)	Signif. *	Standard error	Relative difference between high density and low density
HD	47.98	b	1.08	18.41%
LD	39.15	a	1.08	

Cultivar	Final height (cm)	Signif.*	Standard error
Bicolor	47.15	b	2.02
Pink	42.65	a	2.02
Red	43.28	ab	2.02
Rose	41.28	a	2.02

* Different letters indicate significant differences ($p \leq 0.05$). HD: High Density; LD: Low Density.

* Diferentes letras indican diferencias significativas ($p \leq 0,05$). HD: Alta Densidad; LD: Baja Densidad.

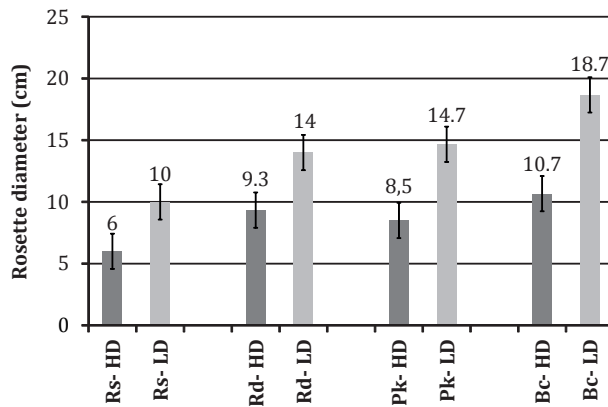


Figure 3. Comparison of rosette diameters (cm) in plants of ornamental cabbage (*Brassica oleracea* L. var. *acephala*) cv Rose (Rs), Red (Rd), Pink (Pk) and Bicolor (Bc) at high planting density (HD) (56 plants/m²) and low planting density (LD) (28 plants/m²). Rosette diameter is the mean value \pm standard error.

Figura 3. Comparación de diámetros de roseta (cm) (media \pm error estándar de la media) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) de los cv Rose (Rs), Red (Rd), Pink (Pk) y Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/m² y baja densidad (BD) 28 plantas/m².

The mean leaf area developed by each plant showed different responses between treatments. In all cases, a lower planting density led to a greater leaf area (figure 4). The mean of the four cultivars showed that the plants grown at low density showed a significant increase in leaf area of 33.2% ($p \leq 0.05$). Regardless of planting density, this variable showed differences between cultivars.

The highest leaf area values were achieved by Red and Bicolor, which, on average, outperformed Pink and Rose with significant differences ($p \leq 0.05$).

The rate of leaf appearance showed a progressive increase as a function of temperature. This relationship was tighter in the treatments at high density (figure 5, page 56). Regarding the root volume per plant, it was found significant differences ($p \leq 0.05$) due to the density

and the cultivar, but not to the density x cultivar interaction (table 2, page 57).

The lower planting density determined a greater root volume (mL/plant) in all the cultivars, with significant differences in Bicolor (figure 6, page 58). Also, the mean of the four cultivars showed that the condition of lower planting density generated a significant increase in this variable with a difference of 39.8%.

Both, AFW and RFW were affected by the density and the cultivar, but not by the interaction between both factors (table 2, page 57). The treatments at low density led, on average, to 34.5% higher AFW, with significant differences ($p \leq 0.05$) regarding the treatments at high density.

The same trend was observed for RFW, where the lower density in each cultivar led to an increase in RFW, with significant differences in Bicolor (figure 7A, page 58).

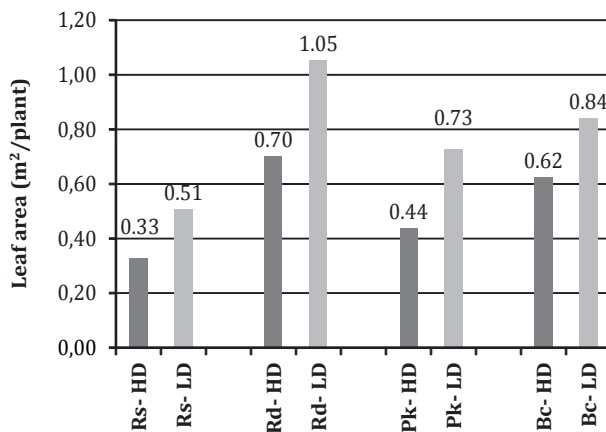


Figure 4. Comparison of the mean leaf area ($m^2/plant$) of ornamental cabbage plants (*Brassica oleracea* L. var. *acephala*) (mean \pm standard error of the mean) cv. Rose (Rs), Red (Rd), Pink (Pk) and Bicolor (Bc) at high planting density (HD) (56 plants/ m^2) and low planting density (LD) (28 plants/ m^2).

Figura 4. Comparación de medias de área foliar ($m^2/planta$) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) (media \pm error estándar de la media) de los cv. Rose (Rs), Red (Rd), Pink (Pk) y Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/ m^2 y baja densidad (BD) 28 plantas/ m^2 .

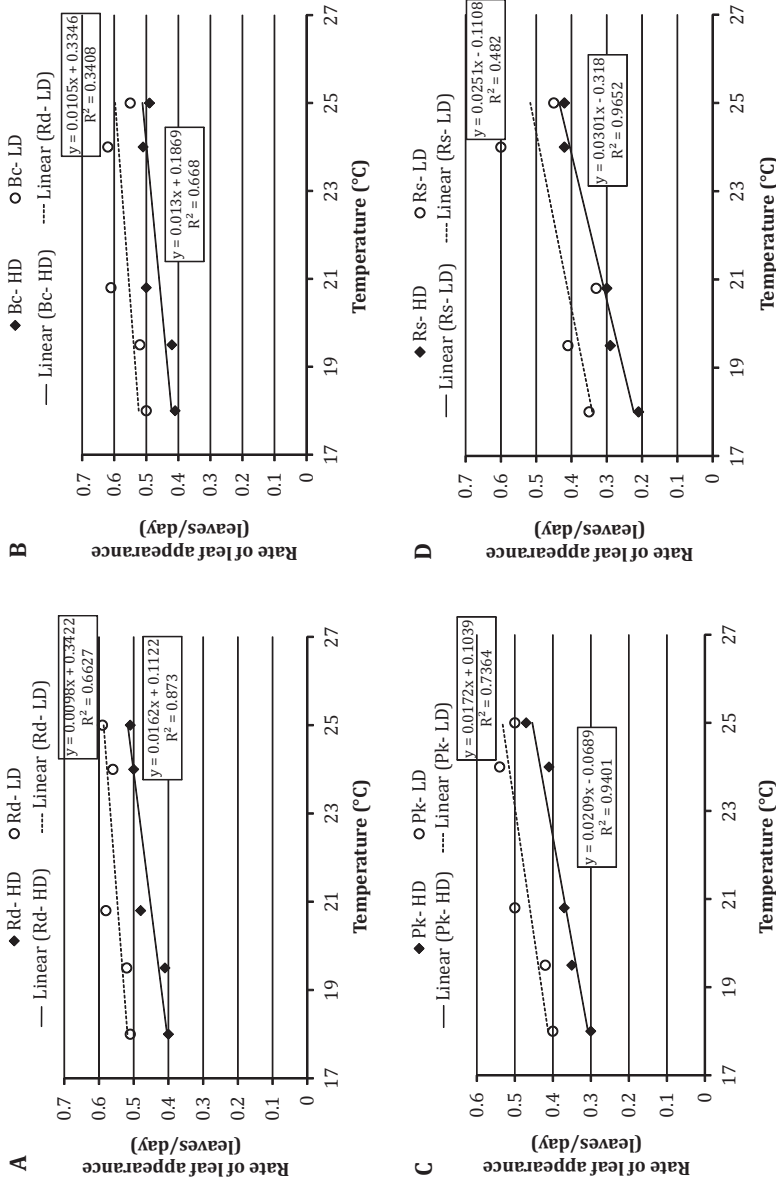


Figure 5. Rate of leaf appearance (leaves/day) in ornamental cabbage plants (*Brassica oleracea* L. var. *acephala*) as a function of the mean daily temperature for the cultivars (A) Rose (Rs), (B) Red (Rd), (C) Pink (Pk) and (D) Bicolor (Bc) at high planting density (HD) (56 plants/m²) and low planting density (LD) (28 plants/m²).

Figure 5. Tasa de aparición foliar (hojas/día) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) en función de la temperatura media diaria para los cv (A) Rose (Rs), (B) Red (Rd), (C) Pink (Pk) y (D) Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/m² y baja densidad (BD) 28 plantas/m².

Table 2. Summary of the results of the ANOVAs and interactions for *B. oleracea* L. var. *acephala* for each of the assessed variables.

Tabla 2: Resumen de los resultados de los ANOVAs y las interacciones para *B. oleracea* L. var. *acephala* en cada una de las variables evaluadas.

Variable	Factor	F	
Rosette diameter	Density	215.3	*
	Cultivar	5.8	*
	D x C	0.1	ns
Leaf area per plant	Density	10.4	*
	Cultivar	6.1	*
	D x C	0.2	ns
Root volume per plant	Density	26.4	*
	Cultivar	6	*
	D x C	0.6	ns
Aerial fresh weight	Density	26.1	*
	Cultivar	14.5	*
	D x C	0.7	ns
Root fresh weight	Density	29.4	*
	Cultivar	7.2	*
	D x C	2.3	ns
Aerial dry weight	Density	24.9	*
	Cultivar	10.5	*
	D x C	0.6	ns
Root dry weight	Density	25.3	*
	Cultivar	7.7	*
	D x C	1.4	ns
Aerial/root fresh weight	Density	0.4	ns
	Cultivar	4.05	*
	D x C	1.48	ns
Aerial/root dry weight	Density	1.59	ns
	Cultivar	1.66	ns
	D x C	0.3	ns

* $p \leq 0.05$; ns: not significant.

* $p \leq 0,05$; ns: no significativo.

On average, the plants grown at low density increased their RFW by 36%, with significant differences ($p \leq 0.05$). Regarding ADW and RDW, there were significant differences due to the planting density and cultivar but not to the density x cultivar interaction (table 2).

The mean of the resulting ADW of the treatments at high density was 33.4% higher, with significant differences ($p \leq 0.05$). The average increase in RDW in the treatments at low density was 37.8%, also with significant differences ($p \leq 0.05$) regarding those at high density (figure 7B, page 59).

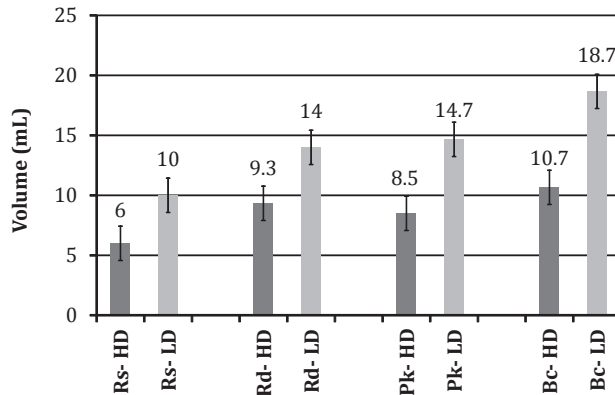


Figure 6. Comparison of means (mean \pm standard error of the mean) of the root volume (mL/plant) in ornamental cabbage plants (*Brassica oleracea* L. var. *acephala*) cv. Rose (Rs), Red (Rd), Pink (Pk) and Bicolor (Bc) at high planting density (HD) (56 plants/m²) and low planting density (LD) (28 plants/m²).

Figura 6. Comparación de medias (media \pm error estándar de la media) del volumen radical (ml/planta) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) de los cv. Rose (Rs), Red (Rd), Pink (Pk) y Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/m² y baja densidad (BD) (28 plantas/m²).

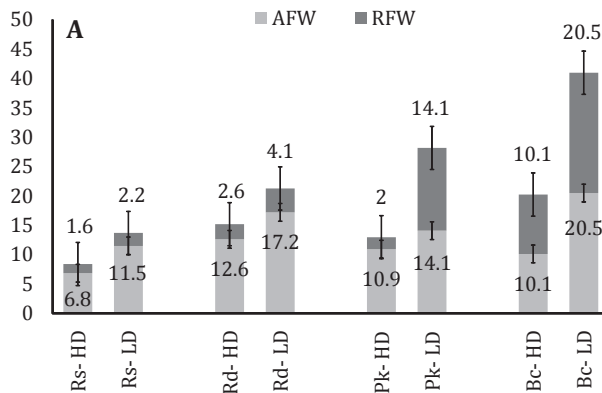


Figure 7. Comparison of means of aerial and root fresh (A) and dry (B) weight (mean \pm standard error of the mean) in ornamental cabbage plants (*Brassica oleracea* L. var. *acephala*) cv. Rose (Rs), Red (Rd), Pink (Pk) and Bicolor (Bc) at high planting density (HD) (56 plants/m²) and low planting density (LD) (28 plants/m²).

Figura 7. Comparación de medias de peso fresco (A) y seco (B) aéreo y radical (media \pm error estándar de la media) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) de los cv. Rose (Rs), Red (Rd), Pink (Pk) y Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/m² y baja densidad (BD) (28 plantas/m²).

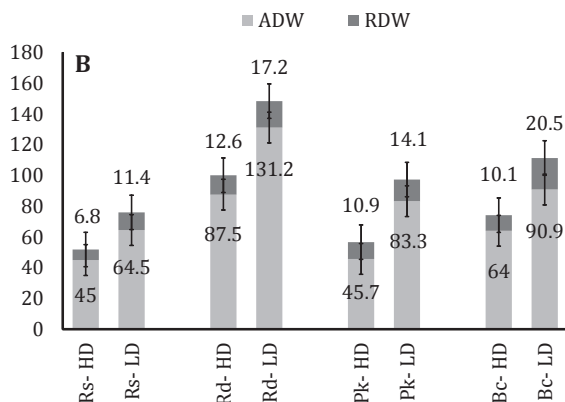


Figure 7. cont. Comparison of means of aerial and root fresh (A) and dry (B) weight (mean \pm standard error of the mean) in ornamental cabbage plants (*Brassica oleracea* L. var. *acephala*) cv Rose (Rs), Red (Rd), Pink (Pk) and Bicolor (Bc) at high planting density (HD) (56 plants/m²) and low planting density (LD) (28 plants/m²).

Figura 7. cont. Comparación de medias de peso fresco (A) y seco (B) aéreo y radical (media \pm error estándar de la media) en plantas de repollo ornamental (*Brassica oleracea* L. var. *acephala*) de los cv Rose (Rs), Red (Rd), Pink (Pk) y Bicolor (Bc) en los tratamientos con plantación a alta densidad (AD) 56 plantas/m² y baja densidad (BD) (28 plantas/m²).

DISCUSSION

The growth and productivity of a crop are the result of the interaction between its genotype and the environment that surrounds it (21).

However, even though the genotype is relatively constant as compared with the environment, its expression is largely influenced by environmental changes and any variable affecting the environment will be reflected in the growth and productivity of the crop (18). Also, as stated by Casal (2013), the management of crops involves decisions on several factors such as the sowing density, defoliation, fertilization, architecture, and choice of cultivar, all of which affect the leaf area and the position of the foliage in relation to the angle of the incoming radiation and therefore the degree of shading.

In this experiment, the planting density notoriously influenced all the parameters evaluated, regardless of the cultivar. A greater planting density led to an increase in plant height probably due to the changes caused in the light environment, *i.e.* due to the decrease in the Red:Far-Red ratio caused by this treatment, generating shade avoidance as a response. This response, the best-known of those mediated by photosensors, leads to a change in the architecture of plants by the elongation of internodes (1).

According to Casal (2013), in dense canopies, upper leaves intercept radiation that would otherwise reach the lower leaves, and plants are mutually shaded. Shade implies the selective attenuation of the wave length of the incident radiation.

Green leaves absorb primarily in the range of UV radiation (280-320 nm for UVB and 320-400 nm for UVA) and in the range of the photosynthetically active radiation (400-700 nm), particularly in the blue and red regions of the spectrum; and transmit and reflect more strongly in the far red (700-800 nm) and infrared regions. According to Smith (1995), the Red:Far-Red ratio provides a highly reliable indication of the degree of shading because it is relatively little affected by other environmental conditions.

The changes in the Red:Far-Red ratio caused by the reflected light constitute an early signal of alert of the presence of neighboring plants, even before the mutual shading of the leaves begins.

In the present study, although the differences in plant height were not expressed clearly from the start of the experiment (may be because the plants had not reached a size large enough to generate competition for the resources of the environment), the stimulus caused by the proximity of neighboring plants could have been early detected by the plants cultivated at high density. Thus, the stem elongation could be attributed to the increased levels of gibberellins (in some cases through inhibition of their inactivation), caused by a low Red:Far-Red ratio (4).

Other researchers also point out that, in these cases, the responses of elongation seem to be based on the combined action of several plant hormones: gibberellins, auxins, brassinosteroids and ethylene (12, 14). Such elongation includes various physiological components and processes that control the extensibility of the cell wall through expansins and xyloglucan endotransglucosylase/hydrolases and by acidification of the cell wall (22, 23). On the other hand, the reduction of the irradiance and the blue/green ratio in dense canopies

would provide signals that do not share all the features of the Red:Far-Red ratio but that are important for the responses of the plants to the shade.

The light spectrum under a dense canopy shows a relative increase in green light (500-580 nm) and a decrease in blue light (400-500 nm).

The green light reflected or transmitted through the surrounding plants can be another signal to detect neighboring plants, because the addition of green light induces the elongation of the hypocotyl in seedlings and the hypoplasia and elongation of the petiole in adult *Arabidopsis* plants (10, 27).

When the canopy closes and the competition between plants is established, additional signals, such as the decline in the photosynthetically active radiation and UV-B light, are generated. These signals can inform plants of the progressive intensity of competition. Similarly to the red light, the UV-B light is absorbed by chlorophyll and used in photosynthesis. Thus, it has been suggested that a relative decrease in UV-B in the light spectrum would also be a signal of detection of neighboring plants and shade.

In the present study, the increase in the height of the plants grown at high density was not accompanied by an increase in the aerial or root biomass. This greater plant height at high density could not be attributed to a differential stem:root partition because no significant differences in fresh or dry weight were found for such relationship between treatments with high and low densities. Numerous studies have shown that the responses of shade avoidance are often accompanied by a reduction in the investment of carbon in organs such as roots and leaves (2, 19).

According to Casal (2013), one of the responses of plants against the limited

generation of photoassimilates under shading is the reduction of potential yield.

In the present study, the higher density of plants also reduced the rosette diameter in the same way in all the cultivars, although the rosette diameters accomplished were within the commercial range (7.5 cm-18 cm).

This results coincide with those reported by Carter (2003), who stated that a smaller spacing between plants promotes the formation of longer stems and reduces the rosette diameter of ornamental cabbages. This same trend has been found in other varieties of the genus *Brassica*. Studies in *B. oleracea* L. var. *capitata* have shown that the diameter of the "heads" decreases as the spacing between plants decreases (25).

In *B. oleracea* L. var. *italica*, it has been found that a high planting density reduces the size and the weight of the floret (6) as well as the diameter of the stem and the yield per plant (7).

In the present study, the cumulative number of new leaves and the leaf area per plant decreased as density increased. This trend was recorded in the four cultivars. Similar results have been previously found by Criollo and García (2009) in radish plants (*Raphanus sativus*) cultivated in pot and in greenhouse, in which treatments with lower density allowed achieving a greater leaf area. As stated by these authors, the increase in population density caused a decrease in size, vigor and weight of the plants.

In this study, the rate of leaf appearance was lower in the treatments at high density than in those at low density, in coincidence with that reported by Jolliffe and Gaye (1995), who claimed that growth is faster and the expansion of leaves is greater when there are no competing plants nearby. However, a much more marked adjustment in the rate of leaf appearance

as a function of the environmental temperature was found in the treatments at high density than in those at low density when the temperatures were between 17 and 27°C (temperatures that promote the active growth of *B. oleracea acephala*).

Temperature is one of the most important physical factors of the environment that influence the processes of growth and development of plants (17). This variable has a widely-accepted role as a main factor in the control of the phyllochron, since it affects different biochemical processes that regulate the meristematic activity, closely associated with the development of the leaf and stem (9, 16). Thus, the value of the adjustments obtained in the treatments at high density would allow hypothesizing that, once the stimulus that induces shade avoidance is captured (due to the modification in the Red:Far-Red ratio of dense canopies), plants could present a greater sensitivity to other environmental factors (in this case temperature) than the plants grown at low density. Also, it should be noted that, as time passed by, plants grown at high density lost the leaves in the lower strata of the crop. It is likely that the increase in density, due to the increase in the shading, generated their senescence and abscission.

Casal (2013) argues that, in the vertical avoidance of shading, the average position of foliage is high due to the increase in the height of the stem and/or a decrease in the foliage of the lower strata. In this way, on average, the foliage is less shaded because the upper layers have more light and the increase in leaf senescence reduces the foliage volume at the base of the canopy.

However, although it is important that plants retain a sufficient number of leaves along their cultivation cycle to ensure the growth through the contribution

of photoassimilates, the abscission of leaves in the lower strata in the treatments at high density could represent a comparative advantage respect to the crop grown at low density, because one of the technical recommendations for crop management consists in removing the lower leaves ("defoliation"), after which plants reach an average height of 25 cm. Unlike that observed in other crops, in ornamental cabbages, the decrease in foliar area per plant (with the consequent reduction in the rosette diameter) and the natural abscission of the leaves of the lower strata of the crop would be effective to improve the visual appearance of the harvested plants. Besides their coloration, one of the main attributes of ornamental cabbages is the compactness of the apical rosette whose appearance resembles that of roses. This is fundamental because, unlike other foliage for cutting that act as accompaniment products or complements in bouquets or flower arrangements, ornamental cabbages for cutting are novel products, being protagonists as "cut flowers" within the bouquet.

The higher values of root volume per plant and leaf area per plant observed in all low density treatments could be attributed to the greater space available per plant, which allowed an increase in the exploration of the soil, thus giving plants a greater capacity of absorption of water and nutrients. This was reflected in the responses of AFW, RFW, ADW and RDW, which showed a clear tendency

to increase with lower planting density (figure 7, page 58-59). These results agree with those of Pérez *et al.* (2005), who reported that high planting densities reduce the growth of vegetative variables such as leaf area, number of leaves and dry matter, being these effects more notorious at the end of the crop.

The differences found between cultivars, for each of the parameters evaluated, are probably due to genetic features, being an indicator of the potential of growth of each plant. In this sense, the cultivars Bicolor and Red showed better performance in most of the variables evaluated, whereas, under the same conditions, Rose reached the lowest values.

CONCLUSIONS

In the cultivars evaluated, the density of plants may be increased as an alternative that would not only allow a more efficient use of the crop, but also improve quality indices, achieving greater plant height and more compact apical rosettes, while reducing crop management activities, such as manual leaves removal, which is made naturally. However, further studies with other cultivars should be carried out to test the effect of different densities under the growth conditions of each cultivar, in order to find the optimal planting density in each case.

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Effect of foliar copper application on yield and anthocyanin concentration in *Hibiscus sabdariffa* calyces

Aplicación foliar de cobre sobre el rendimiento y concentración de antocianinas en cálices de *Hibiscus sabdariffa*

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ABSTRACT

Frequent anthocyanin consumption improves health and prevents diseases due to its antioxidant activity. Exposure of some plants to high concentrations of heavy metals may increase anthocyanin concentration and thus improve their food quality. This study determined the effect of Cu spraying on hibiscus (*Hibiscus sabdariffa* L.) leaves at various dosages and number of doses on anthocyanin content, physical and chemical characteristics, and calyces yield. For this purpose, hibiscus genotype Reina Roja was grown under rainfed conditions. During the vegetative stage, Cu was sprayed two, four or six times with 150, 300 and 450 mg L⁻¹. The results indicate that four and six sprayings with 150, 300 and 450 mg Cu L⁻¹ reduced dry calyces yield. Two sprayings at either Cu dosage did not modify calyces yield. Added Cu increased significantly anthocyanin content and titratable acidity and decreased ascorbic acid content in the calyces. Anthocyanin content increased the most (57 and 44%) when Cu was sprayed six times at 300 and 450 mg L⁻¹. The data suggests that two sprayings with 150 mg Cu L⁻¹ could improve nutritional quality of hibiscus extracts without affecting dry calyces yield.

Keywords

Hibiscus sabdariffa • anthocyanins • ascorbic acid • titratable acidity

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RESUMEN

El consumo frecuente de antocianinas mejora la salud y previene enfermedades, debido a su actividad antioxidante. La exposición de algunas plantas a concentraciones altas de metales pesados puede incrementar la concentración de antocianinas y mejorar su calidad funcional. Este estudio determinó el efecto de la aplicación foliar de Cu en varias dosis y número de aplicaciones sobre el contenido de antocianinas, características físico-químicas y rendimiento de cálices de jamaica (*Hibiscus sabdariffa* L.). Para este propósito se cultivó el genotipo Reina Roja que durante la etapa vegetativa, se asperjó al follaje con Cu en dos, cuatro y seis ocasiones con 150, 300 y 450 mg L⁻¹. Los resultados indican que cuatro y seis aplicaciones de cobre redujeron el rendimiento de cálices secos para todas las dosis. Dos aplicaciones no modificaron el rendimiento de cálices. El Cu incrementó significativamente el contenido de antocianinas, acidez titulable y disminuyó el contenido de ácido ascórbico en los cálices. Los aumentos mayores de antocianinas (57 y 44%) fueron con seis aplicaciones de 300 y 450 mg de Cu L⁻¹. Los datos sugieren que dos aplicaciones de 150 mg de Cu L⁻¹ pueden mejorar la calidad nutracéutica de los extractos de jamaica sin afectar el rendimiento de cálices secos.

Palabras clave

Hibiscus sabdariffa • antocianinas • ácido ascórbico • acidez titulable.

INTRODUCTION

The demand of plant products as functional food staple is increasing due to the direct relationship between diet and health (11, 30). The properties of some plant substances and their possible use in prevention and mitigation of chronic diseases caused mainly by free radicals have been identified (10, 15, 20). Some of the phytochemical compounds with nutraceutical activity are carotenoids, phytosterols, flavones, isoflavones, flavonols, flavonones, stilbenes, phenolic acids, lignans and anthocyanins (15, 18).

Anthocyanins are polyphenols that color leaves, flowers and fruits of red, purple and blue. The main plant sources for anthocyanins are berries like cranberries (*Vaccinium sp.*), blackberries (*Rubus spp.*), raspberries (*Rubus idaeus*) and strawberries (*Fragaria x ananassa* D.), as well as grapes (*Vitis vinifera* L.) and

hibiscus calyces (*Hibiscus sabdariffa* L.) (23, 29). Anthocyanins function as antioxidants, diuretic, antimicrobial, antiobesity, immune modulators, antiurolithiatic, hepatoprotective and hypocholesterolemic (20). Additionally, they reduce cholesterol levels and decrease the risk of acquiring diabetes, cancer, and other diseases (19, 20).

Hibiscus sabdariffa L. is a malvaceae grown mainly in tropical and subtropical regions at Sudan, Taiwan, Thailand and Mexico. In Mexico it is cultivated in approximately 20,055 ha with a production of 7,528 t (25). This crop is made by producers of scarce resources without mechanization, which favors occupation (5). Dry flower calyces are used to prepare tea, wine, cocktails, sauce, jelly, candies and cookie flour; however, its main use is preparation of refreshing beverages

from extracts (20). Its consumption has increased mainly due to promotion efforts that classify it as a functional food (10, 20). Efforts to increase secondary metabolite content, like anthocyanins, in hibiscus calyces could increment health benefits and improve prices for producers. Environmental stresses like high and low temperatures, drought, salinity, UV stress or heavy metals could alter their metabolism and thus the physical and chemical characteristics of the plants (2, 31).

Exposure to heavy metals increases free radical production. Anthocyanins have greater capacity than vitamins C and E to protect the plant against oxidative stress by donation of hydrogen atoms that stabilize unpaired electrons from free radicals. It is then possible to induce secondary metabolite synthesis by stress conditions (27).

Application of As on *Lemna gibba* with 0,25, 0,5, 1,0 and 1,5 mg L⁻¹ increased anthocyanin content; the highest content was found at the highest dosage and produced 45 mg 100 g⁻¹ of fresh biomass (FB) (17).

In *Arabidopsis thaliana* under *in vitro* conditions, exposure to Mn, Cu, Zn, Pb and Hg increased anthocyanin content: Cu with 250 µM increased anthocyanins more efficiently from 2 to 32,5 µg g⁻¹ of fresh biomass (FB). Zn increased the highest (14 µg g⁻¹ FB) with 1000 µM (7). In both studies, the variables measured included chlorophyll content, dry biomass weight, root length and shoot length (7, 17).

According to Leão *et al.* (2014), plants respond differently to stress by heavy metals, and the response depends on the metal, the concentration and the duration of the stress. However, in *Atriplex hortensis* var *Purpurea* and *A. rosea* exposure to different concentrations of Cu, Ni, Pb and Zn did not modify anthocyanin content (22).

Hibiscus calyces respond to cobalt and nickel applications, either separate or combined, by increasing content of anthocyanins and other flavonoids. The highest values (31.7 mg anthocyanins g⁻¹ dry biomass) occurred with 20 mg of Co + 25 mg Ni kg⁻¹: a 96% increment compared to the control. Plant height, number of fruits and calix yield also improved (6). Leaf spraying of heavy metals could improve this response. Cu is sold as leaf fertilizer, is easily purchased, contributes to plant nutrition, and improves crop yield. There are limited number of hibiscus-related studies that explore changes in anthocyanin production and improvements on physical and chemical characteristics of extracts. This study determined the effect of Cu leaf spraying at different dosages and number of doses on anthocyanin content, physical and chemical characteristics of calyces, and calyces yield.

MATERIALS AND METHODS

The experiments were conducted under rainfed conditions in Cajones, Municipality of Gabriel Zamora, Michoacán, México at 19°10'49" N; 101°58'13" and 503 a. m. s. l. The climate is warm sub-humid, with summer rains (AW₀) (13).

On July 2016, Reina Roja hibiscus seeds were handed out to local producers. Two seeds per bush were planted at 0.5 m between each bush and 0.8 m between rows. Population density was 50.000 plants per hectare.

Previous tests determined that Cu leaf spraying should start 15 days after sowing at 150, 300 and 450 mg L⁻¹ of water per spraying (D150, D300, D450). Plants were dosed two, four and six sprayings (A2, A4 and A6) spaced every seven days.

Nine treatment combinations were tested (D150-A2, D150-A4, D150-A6, D300-A2, D300-A4, D300-A6, D450-A2, D450-A4, D450-A6), as well as a control (D0-A0), which were distributed in the field under a randomized complete block experimental design in a split-plot arrangement. The large plot was the dosage and the small plot the number of applications. Each treatment was repeated four times for a total of 40 experimental units. Each unit included three meter long rows with 36 plants.

The Cu source was pentahydrated cupric sulfate applied with adherent agent Inex (1 mL L^{-1}). Leaves were sprayed in the mornings until dripping. The sprayer was 15 L in capacity and included an empty cone tip (hollow).

Crop fertilization included 80 and 40 kg ha^{-1} of N and P_2O_5 to the soil as urea (46-0-0) and triple calcium superphosphate (0-46-0). Half of the N dosage and all the P_2O_5 dosage were applied mixed 15 days after sowing (das); the rest of the N dosage was applied at 45 das.

At plant physiological maturity (147 das) when the fruits started with brown coloration and the seeds get a black coloration the harvest of fruits with calyces was carried with the hands, from the middle row from each experimental unit. Afterwards, the fruit was separated from the calyces with a punch. Subsequently, the number of calyces per plant were counted, and their diameter and length recorded. Calyces were stored in paper bags and dried in a forced circulation oven at 50°C until constant weight was achieved. After drying, the weight of 10 calyces was recorded, as well as dry calyces yield.

From each experimental unit, 15 g of dry calyces were ground in an IKA mill

model MF 10 equipped with a 0.5 mm screen. From each sample, duplicate water extracts were prepared with 2,5 g of powder each. The extract was transferred to 250 mL Schott Duran glass vials, and 200 mL of distilled water were added. The mixture was left untouched for 24 h at ambient temperature in the dark. The following day, the extracts were placed in a water bath at 40°C for 15 minutes.

The following variables were measured in the extracts: pH with a Beckman pH Meter (method 981.12); total soluble solids with an ATAGO digital refractometer (0 to 32% range) (method 932.12); titratable acidity as percentage of citric acid (method 942.15); and ascorbic acid content with 2, 6 dichlorophenol indophenol (method 967.21). The determinations were made according to the methodology of the Association of Official Analysis Chemistry (4).

Anthocyanin content was determined with the Abdel-Aal and Hucl method (1999). This method required 100 mg of powder from each ground sample. This sample was added to centrifuge tubes and 10 mL of extraction solution [acidified methanol (methanol: HCl 1,5N in an 85:15 v/v ratio)].

The mixture was kept in the darkness for 24 h at 4°C . After this period, the tubes were centrifuged at 5000 rpm for 20 minutes in a Heraeus Biofuge Primo R. Samples were then diluted to a 1:10 ratio (100 μL of concentrated extract + 900 μL of acidified methanol) and their concentration determined in a Genesys 10 UV (Termo Spectronic) spectrophotometer at 533 nm with extraction solution as blank.

Anthocyanin content was estimated as equivalents of cyanidine-3-glucoside using the equation:

$$A = (Ab/\epsilon)(Ve/1000)MW(1/Ph)(10^6)$$

where:

A = anthocyanin content

Ab=the absorbance for the sample,

sigma= molar extinction coefficient for cyanidine-3-glucoside (25965 cm⁻¹ mol⁻¹)

Ve = total extract volume

MW = the molecular weight for cyanidine-3-glucoside (449)

Ph = sample weight

Total anthocyanin content was recorded in mg of anthocyanins per g of dry calyces biomass.

Data was analyzed using ANOVA and mean comparison among treatments (Tukey ≤ 0.05) in SAS version 9.0 (24).

During the development of the crop the maximum temperature, minimum temperature and rainfall were recorded.

On average, the maximum and minimum temperatures were 33.5°C and 17.7°C, respectively with accumulated precipitation of 367.9 mm (figure 1).

RESULTS AND DISCUSION

Calyces yield and yield components

Supplied Cu in two applications at either dosage did not modify statistically calyces yield: yields for 150, 300 and 450 mg L⁻¹ were similar to yields produced by untreated plants (1272 kg ha⁻¹).

The 300 mg L⁻¹ dosage produced heavy calyces, 12.8% heavier than the control, but it did not improve yield because the number of calyces per plant decreased (table 1, page 70).

Cu spraying reduced significantly (P ≤ 0.01) calyces yield when applied four and six times in all dosages; it significantly decreased calyces length, calyces diameter, number of calyces per plant and calyces weight (table 1 and 2, page 70).

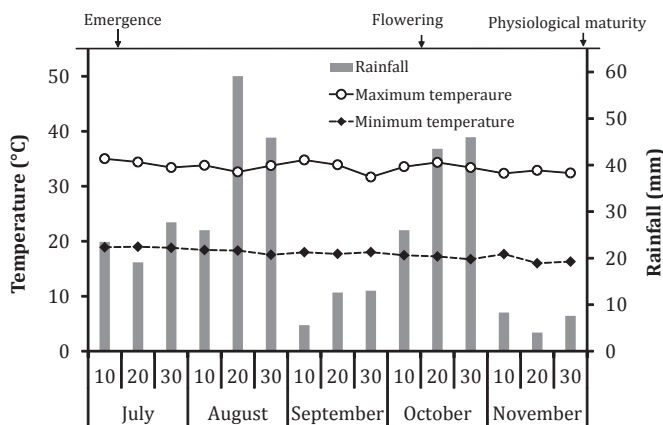


Figure 1. Rainfall distribution (ten days sum), maximum and minimum temperature (ten days average) during crop growth.

Figura 1. Distribución de la precipitación pluvial (suma decenal), temperatura máxima y mínima (promedio decenal) durante el crecimiento del cultivo.

Table 1. Dry calyces yield (DCY), calyces diameter (CD), calyces length (CL), number of calyces per plant (NC), weight of 10 calyces (W10C) of *Hibiscus sabdariffa* in response to dosage and number of foliar Cu applications. Tukey means comparisons.

Tabla 1. Rendimiento de cálices secos (DCY), diámetro de cálices (CD), longitud de cálices (CL), número de cálices por planta (NC) y peso de 10 cálices (W10C) de *Hibiscus sabdariffa* en función de la dosis y número de aplicaciones foliares de Cu. Comparación de medias de T.

Cu dosage (mg L ⁻¹)	Number of doses	DCY (kg ha ⁻¹)	CD (cm)	CL (cm)	NC	W10C (g)
0	0	1272.1 a	3.22 a [†]	4.32 a	33.8 ab	7.65 b
150	2	1299.2 a	3.17 ab	4.16 abc	36.3 a	7.2 bc
	4	1061.1 c	3.07 ab	3.91 c	24.3 c	7.68 b
	6	1045.9 c	3.16 ab	4.15 abc	32.8 ab	6.48 d
300	2	1245.4 ab	3.14 ab	4.18 ab	29.0 bc	8.63 a
	4	1067.5 c	2.81 c	3.57 d	29.3 bc	7.35 bc
	6	1069.2 c	3.19 ab	4.27 a	30.8 ab	7.03 bcd
450	2	1244.2 ab	3.09 ab	3.95 bc	35.5 a	7.03 bcd
	4	928.4 c	3.13 ab	4.14 abc	29.3 bc	6.98 cd
	6	1087.5 bc	3.01 b	4.16 abc	29.0 bc	7.53 bc
General Mean		1132.0	3.1	4.08	30.1	7.45
MSD _{0.05}		168.0	0.18	0.25	6.16	0.65
CV		6.10	6.32	8.50	7.9	9.40

[†] Means with the same later within each column are not statistically different (Tukey, P ≤ 0.05). MSD_{0.05} = minimum significant difference at 5% error probability. CV = coefficient of variation.

[†] Medias con la misma letra dentro de cada columna no son diferentes estadísticamente (Tukey, P ≤ 0,05). MSD_{0,05} = diferencia mínima significativa al 5% de probabilidad de error. CV = coeficiente de variación.

Table 2. Level of significance for dry calyces yield (DCY), calyces diameter (CD), calyces length (CL), number of calyces per plant (NCP) and weight of 10 calyces (W10C) in *Hibiscus sabdariffa* in response to Cu dosage, number of leaf sprayings and the interaction dosage x doses.

Tabla 2. Nivel de significancia para rendimiento de cálices secos (DCY), diámetro de cálices (CD), longitud de cálices (CL), número de cálices por planta (NCP) y peso de 10 cálices (W10C) en *Hibiscus sabdariffa* en función de la dosis de Cu, número de aplicaciones foliares y la interacción dosis x número de aplicaciones.

Factor	DCY	CD	CL	NCP	W10C
Dosage (D)	ns	ns	**	*	ns
Doses (A)	**	**	**	**	**
D x A	**	**	**	**	**

*, ** P ≤ 0.01 y 0.05, respectively. ns = not significant.

*, ** P ≤ 0,01 y 0,05, respectivamente. ns = no significativo.

Plants sprayed four times with 300 mg L⁻¹ per application produced the smallest calyces: 12.7% smaller diameter than the control treatment. The 150 mg L⁻¹ treatment applied four times registered the lowest number of calyces per plant: 28.1% lower number and 9.5% shorter calyces.

Results from the statistical analysis showed that dry calyces yield and yield components were most affected by the number of Cu applications rather than dosage (table 2, page 70). According to the interaction dosage (D) * number of doses (N) it was found that the increase in the number of copper applications at any dose reduced the yield of dry calyces and the values of the yield components. It should be noted that this effect was not proportional in all doses of Cu, to be less the dejection with 300 mg L⁻¹ (table 1 and 2, page 70).

Reduction of calyces yield by Cu sprayings could be attributed to application of higher than recommended concentrations for crops according to Fageria *et al.* (2009). Plant exposure to high heavy metal dosages could cause toxicity, but it is also possible that plants develop mechanisms to tolerate stress.

The mechanisms that stand out are metal exclusion, metal accumulation and metal union to cysteine rich proteins. Plants can also promote the antioxidant system to fight oxidative lesion induced by heavy metals (3, 9). This behavior might explain how calyces yield did not change with two Cu sprayings at 150, 300 and 450 mg L⁻¹ dosages.

Calyces yield reduction in hibiscus with four and six Cu sprayings for all dosages tested might respond to repeated and prolonged stress to the plant with these treatments. Previous research mentions this type of stress catalyzes the production

of oxygen reactive species like superoxide, hydrogen peroxide and hydroxyl radicals that damage cellular components like DNA, proteins and lipids and affect crop growth and yield (28).

Similar response was found with a single application of ZnSO₄ at 2000 µM and CdCl₂ at 250 µM on genotypes Drakkar, Cossair and Pactol of *Brassica napus* L. then reduced significantly dry biomass production (14). Likewise, in rice (*Oryza sativa* L.) 100 µM of Cu caused significant reductions in growth and dry biomass accumulation (28).

A positive effect was found in soil-grown hibiscus treated with Co (20 and 40 mg Kg⁻¹) and Ni (25 and 50 mg Kg⁻¹), alone or in combination. These elements improved up to 137% fresh and dry calyces yield by significantly increasing the number of calyces per plant (6). This response, which is opposite to the response found in this study, might depend on the differential response of plants to heavy metals: their response depends on type of metal, application mode, concentration, and length of stress (8, 17).

Physical and chemical characteristics of hibiscus extracts

Cu spraying for all the treatments reduced significantly ($P \leq 0.01$) ascorbic acid content in all calyces. The highest reduction (41.5%) occurred with two sprayings at 300 mg Cu L⁻¹. Four sprayings at 450 mg Cu L⁻¹ caused the lowest reduction (19.4%) (table 3 and 4, page 72).

Regarding D * N, it was found that the reduction of ascorbic acid with the increase in the number of applications was not proportional in all the doses of Cu evaluated, when there was greater abatement with 150 mg Cu L⁻¹, followed by 300 mg L⁻¹ (table 3 and 4, page 72).

Table 3. Ascorbic acid (AA), titratable acidity (TA), pH, soluble solids (SS) and anthocyanin content in *Hibiscus sabdariffa* calyces in response to dosage and number of Cu leaf doses. Tukey means comparisons.

Tabla 3. Ácido ascórbico (AA), acidez titulable (TA), pH, sólidos solubles (SS) y contenido de antocianinas en cálices de *Hibiscus sabdariffa* en función de la dosis y número de aplicaciones de Cu. Comparación de medias de Tukey.

Cu dosage (mg L ⁻¹)	Number of doses	AA (mg 100 g ⁻¹)	AT (%)	pH	SS (°Brix)	Anthocyanin (mg g ⁻¹)
0	0	85.5 a [†]	10.5 b	2.94 a	4.32 a	18.9 d
150	2	57.6 d	12.2 a	2.85 a	4.18 a	24.8 bc
	4	56.1 de	10.5 b	2.94 a	4.31 a	24.3 bc
	6	59 d	12.2 a	2.84 a	4.17 a	23.4 c
300	2	50 e	11.7 ab	2.91 a	4.27 a	24.7 bc
	4	61.8 cd	10.8 ab	2.96 a	4.34 a	24.3 bc
	6	58 d	12 ab	2.92 a	4.29 a	26.9 a
450	2	57.6 d	10.8 ab	2.91 a	4.27 a	24.7 bc
	4	67.5 bc	12 ab	2.87 a	4.22 a	25.7 ab
	6	68.9 b	12.2 a	2.86 a	4.20 a	27.3 a
General Mean		62.2	11.5	2.90	4.26	24.5
MSD _{0.05}		6.5	1.7	0.17	0.25	1.97
CV		4.7	4.2	2.4	2.4	3.30

[†] Means with the same later within each column are not statistically different (Tukey, $P \leq 0.05$).

MSD_{0.05} = minimum significant difference at 5% error probability. CV = coefficient of variation.

[†] Medias con la misma letra dentro de cada columna no son diferentes estadísticamente (Tukey, $P \leq 0,05$).

MSD_{0,05} = diferencia mínima significativa al 5% de probabilidad de error. CV = coeficiente de variación.

Table 4. Level of significance for ascorbic acid (AA), titratable acidity (TA), pH, soluble solids (SS) and anthocyanins (ANT) in *Hibiscus sabdariffa* calyces in response to Cu dosage, number of leaf doses and the interaction dosage x doses.

Tabla 4. Nivel de significancia para ácido ascórbico (AA), acidez titulable (TA), pH, sólidos solubles (SS) y antocianinas (ANT), en cálices de *Hibiscus sabdariffa* en función de la dosis de Cu, número de aplicaciones y la interacción dosis x número de aplicaciones.

Factor	AA	TA	pH	SS	ANT
Dosages (D)	**	**	ns	ns	**
Doses (A)	**	**	ns	ns	**
D x A	**	**	ns	ns	**

*, ** $P \leq 0.01$ y 0.05 , respectively. ns = not significant.

*, ** $P \leq 0,01$ y $0,05$, respectivamente. ns = no significativo.

The percentage of titratable acidity increases with most treatments: the highest values were registered with two and six sprayings at 150 mg Cu L⁻¹, and six sprayings at 450 mg Cu L⁻¹. Both treatments were statistically similar, and acidity was 16% higher.

The statistical analysis indicated that number of sprayings was the determinant factor in increasing titratable acidity since in most dosages six sprayings produced the highest increments (table 3 and 4, page 72). Interaction was found between D * N, which indicates that the increase in the number of applications of Cu caused higher rates of increase in the titratable acidity of the calyces of Jamaica with the dose of 450 mg Cu L⁻¹, followed by 300 mg Cu L⁻¹.

The increase in titratable acidity percentage is important because it carries a bactericide effect, favors assimilation of metallic ions in the body, acts against formation of low solubility salts, and promotes the freshness sensation when ingesting hibiscus-based drinks (21).

Starting at two Cu sprayings, any of the tested dosages increased significantly ($P \leq 0.01$) anthocyanin content in hibiscus calyces. Two 150 mg L⁻¹ sprayings increased anthocyanin content by 31%. This response was stronger with six sprayings at 300 and 450 mg Cu L⁻¹: mg of anthocyanins g⁻¹ of dry biomass increased by 42 and 44 %, respectively (26,9 and 27.3). According to the interaction D * A the increase in the number of applications of Cu caused greater increases in the content of anthocyanins with 450 mg L⁻¹ followed by 300 mg Cu L⁻¹ (table 3 and 4, page 72).

Anthocyanins are secondary metabolites that protect plants against oxidative stress caused by diverse factors, including exposure to high concentrations of heavy metals. Under these conditions, plants might increase anthocyanin content (6), as the results in this study indicate from two sprayings at 150 mg L⁻¹. Increments in anthocyanin content due to exposure to high concentrations of heavy metals and semi-metals has been documented in other crops: in gibbous duckweed (*Lemna gibba* L.) by exposure to arsenic (17); in *Arabidopsis thaliana* under *in vitro* conditions by addition to the growth media of Cu, Zn, Mn, Pb and Hg (7); in *Capsicum annum* L. by leaf spraying of Cu and Zn to young leaves (26); and in greenhouse-grown Hibiscus supplied at the soil with Co (20 and 40 mg Kg⁻¹), Ni (25 and 50 mg Kg⁻¹), and their combinations (6).

The study shows that foliar Cu applications improves anthocyanin content as well as titratable acidity percentage, but decreased ascorbic acid content. These results are like those reported by Salinas-Moreno *et al.* (2012): they observed that ascorbic acid content in hibiscus calyces is inversely proportional to anthocyanin concentration. Even though ascorbic acid has antioxidant activity, it has been shown that its activity is lower than that of anthocyanins (16, 27).

All Cu treatments improved anthocyanin content in hibiscus calyces. This polyphenol is highly important because of its beneficial properties on health when consumed (19, 20). Four and six sprayings at 150, 300 and 450 mg L⁻¹ increased anthocyanin content the highest, but dry calyces yield decreased. Thus, two sprayings at any of the highest dosages could improve anthocyanin content without reducing calyces yield.

CONCLUSIONS

Twice Cu application with 150, 300 and 450 mg L⁻¹ did not modify caly yield, but four and six applications reduced yield by decreasing calyx size, weight and number per plant. Added Cu increased anthocyanin content and percentage of titratable acidity and decreased ascorbic acid concentration in hibiscus calyces.

The response was higher with four and six Cu sprayings in the three dosages applied. pH and soluble solids in Hibiscus calyces did not change in response to applied Cu. The treatments that increased anthocyanins content without affecting yield were two Cu applications with 150, 300, 450 mg L⁻¹.

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Agronomic and economic efficiency of the decanted phosphate as function of the liming in marandu grass (*Brachiaria brizantha*)

Eficiencia agronómica y económica del fosfato decantado como función del encalado en hierba marandu (*Brachiaria brizantha*)

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Nota científica

ABSTRACT

The decanted phosphate (DP) is an environmental liability for the fertilizer industries and its use as a phosphate fertilizer has been suggested in agriculture. However, there are few researches that studies its efficiency. In this sense, this experiment was conducted in a greenhouse with the main objective to evaluate the agronomic efficiency and the relative economic efficiency of decanted phosphate (DP) in the presence and absence of lime during three growth periods of *Brachiaria brizantha* cv. Marandu (marandu grass). In addition, the critical levels of P were determined in soil and shoots of plants. The indices Equivalent Triple Superphosphate medium (EqTS medium) e Relative Economic Efficiency (REE) were calculated in order to evaluate the efficiency of DP and triple superphosphate (TS). The critical levels of P were set when relative production was 90%. The DP efficiency indices were more than 100% when not applied liming. The liming reduces the agronomic and economic efficiency of DP. The P critical levels in soil and shoots of the marandu grass were 15 mg dm⁻³ and 1.2 g kg⁻¹, respectively.

Keywords

pH • phosphorus • residual effect • critical level of P. *Brachiaria brizantha*.

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RESUMEN

El fosfato decantado (FD) es una responsabilidad ambiental para las industrias de fertilizantes y se ha sugerido su uso como fertilizante de fosfato en la agricultura. Sin embargo, hay pocas investigaciones que estudien su eficacia. En este sentido, este experimento se realizó en un invernadero con el objetivo principal de evaluar la eficiencia agronómica y la eficiencia económica relativa del fosfato decantado (FD) en presencia y ausencia de cal durante tres períodos de crecimiento de *Brachiaria brizantha* cv. Marandu (hierba marandu). Los niveles críticos de P se determinaron en el suelo y los brotes de las plantas. El índice equivalente superfosfato triple medio (EqST medio) y la eficiencia económica relativa (EER) se calcularon para evaluar la eficacia de FD y superfosfato triple (ST). Los niveles críticos de P se establecieron cuando la producción relativa fue del 90%. Los índices de eficiencia del FD fueron más del 100% cuando no se aplicó el encalado. El encalado reduce la eficiencia agronómica y económica de FD. Los niveles críticos de P en el suelo y en la parte aérea de la hierba marandu fueron 15 mg dm^{-3} y $1,2 \text{ g kg}^{-1}$, respectivamente.

Palabras clave

pH • fósforo • efecto residual • nivel crítico de P. *Brachiaria brizantha*.

INTRODUCTION

Studies suggest the establishment of agronomic and economic indices for the choice of the phosphate fertilizer (3, 8, 10). In this sense, the equivalent triple superphosphate medium (EqTS medium) and Relative Economic Efficiency (REE) are more interesting than other commonly used indices such as Agronomic Efficiency Index (AEI) and triple superphosphate equivalent (EqTS), because it does not suffer the influence of the dose used due to it considers the part of the curves of greater response to the P addition.

Furthermore, the evaluation of P sources should also consider the residual effect of fertilizers, because forms of P may be more or less available for plants over time (11).

The most commonly used P sources are soluble phosphates in water, thermophosphate and natural phosphates. However, these forms have higher prices due to

the dependence on imports. In addition, tropical soils are mostly weathered, acidic and with high adsorption of P to oxides and hydroxides of iron (Fe) and aluminum (Al), making it unavailable (2). This combination increases the search for new alternatives to supply the requirement of crops with this nutrient, and increase the sustainability of agricultural systems.

In this context, the decanted phosphate (DP) can be a possible source of P. The DP is a by-product of wastewater treatment of phosphoric acid production (H_3PO_4) and it is obtained by precipitation reaction of H_3PO_4 diluted (contained in the precipitation ponds) with calcium hydroxide or lime, resulting in a powder that is dried and marketed. This product is an environmental liability and it generates costs for the fertilizer manufacturers, which seek an appropriate destination for this material. In addition, there is a

growing worldwide concern for the reuse of wastewater from industries, especially when they can provide nutrients such as P to soils, reducing depletion of phosphate reserves and avoiding watercourses contamination (13, 14).

The DP has been indicated by companies mainly for pasture due to its initial and residual effect. It is also known that most of the Brazilian pastures present some degradation, mainly related to the deficiency of P in their soils with little corrected acidity (4). In this sense, liming and phosphate fertilization are the most expensive practices in the implementation of a pasture. However, the efficiency of P sources is influenced by changes in the soil pH value (they modify the adsorption reactions of P) and by the chemical composition, solubility and accompanying cation (they interfere in phosphate solubilization) (2, 12).

Moreover, the critical levels of P in the soil defined for fodder were established on the basis of annual crops, with scarcity of information for crops such as *B. brizantha*, which has been included in various agrosystems either in exclusive cultivation or in a consortium with cereals, grains and forest.

As there are few researches that studied efficiency indices of the DP and their relationship with the soil acidity, the main objective of this study was to evaluate the agronomic and relative economic efficiency of the DP in the presence and absence of lime during three consecutive growths periods of *B. brizantha* cv. Marandu (initial and residual effect).

Furthermore, due to the fact that the soil fertility classes used for fodder had been established for annual crops, the present study also aimed to determine the critical levels of P in soil and shoots of the plants.

MATERIALS AND METHODS

Location, period and soil characterization

This experiment was conducted in a greenhouse from October 2012 to January 2013 at Sao Paulo State University - Jaboticabal/SP - Brazil in pots containing samples of sandy clay loam Typic Haplustox.

The soil had the following granulometric characteristic:

clay = 260 g kg⁻¹; silt = 30 g kg⁻¹ and sand = 710 g kg⁻¹.

The chemical attributes of soil fertility were:

pH (CaCl₂) 4.1
 MO = 23 g dm⁻³; missing N content
 P (resin) = 4 mg dm⁻³
 K = 0.4 mmol_c dm⁻³
 Ca = 7 mmol_c dm⁻³
 Mg = 3 mmol_c dm⁻³; S = 4 mg dm⁻³
 H + Al = 58 mmol_c dm⁻³
 CTC = 68 mmol_c dm⁻³ and V = 15%

The experimental design and treatments

The experimental design was completely randomized (with three replications), according to a factorial 6 x 2 x 2 [six doses of P; two sources of P; presence (P/L) and absence of lime (A/L)], totaling 72 experimental units (pot with 2.8 kg of soil).

The doses of P (calculated by total P of the fertilizers) were 0, 30, 60, 90, 120 and 150 mg kg⁻¹ of P.

The P sources were decanted phosphate (DP) (P₂O₅ total = 17%; P₂O₅ soluble in neutral ammonium citrate (NAC) + water = 9%; P₂O₅ soluble in citric acid = 12%; Ca = 18%) and triple superphosphate (TS) (P₂O₅ total = 45%, P₂O₅ soluble in water = 37%; P₂O₅ soluble in NAC + water = 43%; Ca = 14%).

Soils with the acidity correction received lime (PRNT = 95%), in a amount calculated to elevate soil base saturation to 60%. The sources of P were applied in powder form in order to avoid the effects of granulometry and facilitate the mixture of them with the soil. The marandu grass (*Brachiaria brizantha* cv. Marandu) was used as test plant.

Experimental conduction

The lime was mixed with the soil in each pot, followed by the addition of distilled water to the soil to reach approximately 80% of the maximum water retention (MWR).

The soil was incubated for 20 days. The soil in each pot was air-dried and it received phosphate fertilizers and basic fertilization.

The basic fertilization was consisted of doses of N, S, K and Zn in mg kg⁻¹: N = 30 and S = 36 (ammonium sulfate), K = 120 (potassium chloride) = 3 and Zn (zinc sulfate). The sources of P were applied only before sowing.

Marandu grass sowing was made with 30 seeds per pot. Seven days after the plant emergence, thinning was done leaving four plants per pot.

After 20 days of plant emergence, a standardization cut to 0.10 m above ground level was done. Then the fertilization was performed with 120 mg kg⁻¹ N (ammonium sulfate).

After the standardization, three cuts of the shoot were done at intervals of 30 days, also to 0.10 m. After each cut, fertilization was performed with 150 mg kg⁻¹ N (ammonium nitrate) and 80 mg kg⁻¹ K (potassium chloride). Daily waterings with distilled water were made to maintain the soil close to 80% of the MWR.

The shoots at each of the three cuts were washed. The plant materials were dried at 65°C in forced-air oven. The plant material was weighed to determine the dry mass of shoots.

Agronomic and economic efficiency

To evaluate the efficiency of P sources was used the response curve of TS Goedert *et al.* (1986).

The response curves were adjusted following the model $y = y_0 + ax^{0.5}$, and the significance of the regressions were verified ($p < 0.01$).

The comparative analysis of response curves (EqTS medium) was calculated through the quotient squared of the angular coefficients of the curve, multiplied by a hundred, using the DP as numerator (y_2) and TS as divisor (y_1):

$$EqTS\ medium(\%) = \left[\frac{b(y_2)}{b(y_1)} \right]^2 \cdot 100$$

The relative economic efficiency (REE) was obtained by division of the price in reais of one kilogram (kg) of P₂O₅ of TS (PTS), reference source, and the cost of a kilogram of P₂O₅ of test source- DP(PDP), multiplied by the agronomic efficiency (EqTS medium) of the test source and by a hundred (6).

The information for calculating the REE were: the price in real of the fertilizer per ton (t) (DP = R\$ 320/t; TS = R\$ 1,615/t), the total content of P₂O₅ in the fertilizer (DP = 17%; TS = 45%), the PDP = R\$ 1.88, the PTS = R\$ 3.59 and the ratio PTS/PDP = 1.90. Commercial fertilizer prices were obtained on February 24th, 2016.

$$REE(\%) = (EqTS\ medium \cdot \frac{PTS}{PDP}) \cdot 100$$

P critical level

The P critical levels were set when relative production was 90%. The linear plateau model was performed to determine the P critical level in soil and shoots of marandu grass, which was defined by the intersection of the two lines (1).

RESULTS

The adjusted equations that best described the response curves according to each P source, where x is the dose of P used, and y_1 and y_2 correspond to the dry matter production of marandu grass shoots provided respectively by TS and DP (table 1). Through the EqTS medium (table 2, page 82), it was found that the sum of the growths, the DP was more efficient compared to TS when not applied lime.

On the other hand, the DP was less efficient when the soil received this corrective. In the three growths, the EqTS medium provided by the DP were similar or above those verified for the TS when the treatments did not receive lime (EqTS medium ≥ 100).

When the soils received lime, the EqTS medium for DP in the first growth was the lowest of the experiment. This indice was raised to higher values than the ST in the second growth and again reduced to lower after this growth.

Through the REE (table 2, page 82), it can see that the REE indices obtained for the DP were always higher than 100%, except for the first growth when the DP had REE lower than 100% when applied lime to the soil. However, in the sum of the growth, REE for DP was than 100% even when the soil received lime.

Table 1. Response curves for fertilizers as function of the liming in the three growth periods of *Brachiaria brizantha* cv. Marandu and the sum of the growths.

Tabla 1. Curvas de respuesta para fertilizantes en función del encalado en los tres períodos de crecimiento de *Brachiaria brizantha* cv. Marandu y la suma de los crecimientos.

Growths	P sources	Liming	Adjusted equations	R ²
1 ST Growth	Triple superphosphate	Ausence	$y_1 = 0.9947 + 0.5562x^{0.5}$	0.81**
		Presence	$y_1 = 0.0023 + 0.4141x^{0.5}$	0.87**
	Decanted phosphate	Ausence	$y_2 = 1.0186 + 0.5597x^{0.5}$	0.68**
		Presence	$y_2 = -0.1830 + 0.2626x^{0.5}$	0.46**
2 ND Growth	Triple superphosphate	Ausence	$y_1 = 2.1772 + 0.7493x^{0.5}$	0.75**
		Presence	$y_1 = 1.8723 + 0.8825x^{0.5}$	0.80**
	Decanted phosphate	Ausence	$y_2 = 1.8821 + 0.8226x^{0.5}$	0.80**
		Presence	$y_2 = 0.9461 + 0.8956x^{0.5}$	0.89**
3 RD Growth	Triple superphosphate	Ausence	$y_1 = 0.1660 + 0.4467x^{0.5}$	0.90**
		Presence	$y_1 = 0.3223 + 0.6234x^{0.5}$	0.76**
	Decanted phosphate	Ausence	$y_2 = 0.9486 + 0.4471x^{0.5}$	0.78**
		Presence	$y_2 = 0.1048 + 0.4746x^{0.5}$	0.79**
Sum of the growths	Triple superphosphate	Ausence	$y_1 = 3.3380 + 1.7521x^{0.5}$	0.87**
		Presence	$y_1 = 2.1969 + 1.9200x^{0.5}$	0.91**
	Decanted phosphate	Ausence	$y_2 = 3.8494 + 1.8295x^{0.5}$	0.83**
		Presence	$y_2 = 0.8679 + 1.6364x^{0.5}$	0.91**

** significant at 1% probability. / ** significativo al 1% de probabilidad.

Table 2. The equivalent triple superphosphate medium (EqTS medium) and relative economic efficiency (REE) for the three growth periods and sum of the growths of *Brachiaria brizantha* cv. Marandu.

Tabla 2. El equivalente superfosfato triple medio (medio EqTS) y la eficiencia económica relativa (REE) para los tres períodos de crecimiento y la suma de los crecimientos de *Brachiaria brizantha* cv. Marandu.

P source	1 ST Growth		2 ND Growth		3 RD Growth		Sum of growths	
	Liming		Liming		Liming		Liming	
	Ausence	Presence	Ausence	Presence	Ausence	Presence	Ausence	Presence
-----Equivalent triple superphosphate medium (%)-----								
Decanted phosphate	101	40	120	103	100	58	109	73
Triple superphosphate	100	100	100	100	100	100	100	100
-----Relative economic efficiency (%)-----								
Decanted phosphate	192	76	228	196	190	110	207	139
Triple superphosphate	100	100	100	100	100	100	100	100

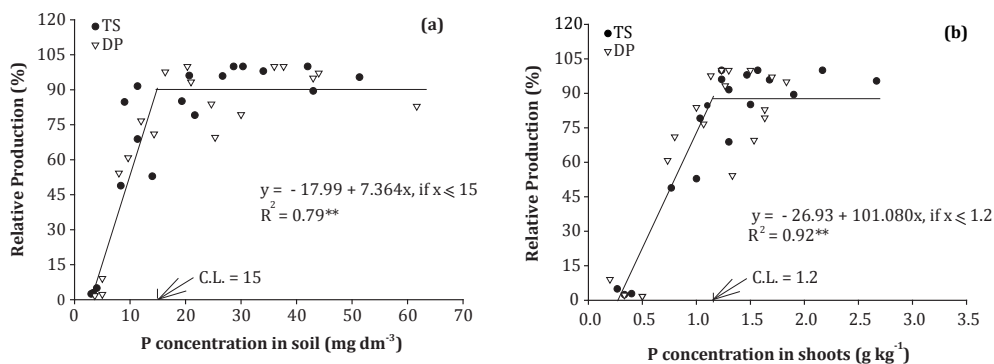


Figure 1. Critical levels of P in soil (a) and shoots of *Brachiaria brizantha* cv. Marandu (b).
Figura 1. Niveles críticos de P en el suelo (a) y brotes de *Brachiaria brizantha* cv. Marandu (b).

The relation between the relative production and the P levels in the soil and shoots of marandu grass is presented (figure 1). The determined critical levels of this nutrient in soil and shoots of the plant were 15 mg dm⁻³ and 1.2 g kg⁻¹, respectively.

DISCUSSION

The P solubilization from the DP had been favored by the higher acidity of the soil, which it was possibly increased throughout the experiment due to nitrogen fertilization performed in each

marandu grass cut. In this sense, other authors verified the efficiency of partially acidulated phosphate was directly proportional to the acidification rate (7).

The values of EqTS medium for DP are higher than those verified by other researchers in field condition for two years for P sources such as magnesium thermophosphate and Gafsa phosphate (90% and 48%, 91% and 88%, respectively) (3).

Moreover, the phosphate fertilizers were applied in powder form, which may also have reduced faster the efficiency of TS (more soluble source in water) after the first growth due to greater surface contact and adsorption P to the soil, reducing the availability of this nutrient.

These results suggest that DP efficiency can be reduced in soils with lower acidity due to the smaller supply of protons H^+ for the solubilization of P from this source and it can be increased in soils with higher acidity. On the other hand, although the increase in acidity could result in better utilization of P from DP, it can also enhance adsorption reactions of this nutrient to the soil that make the EqTS medium had been reduced after the second growth. In acidic soil conditions, positive charges are generated which increase adsorption the phosphate anions to adsorbent soil surface.

In addition, precipitation reactions of P can also occur with ionic forms of Al and Fe in acidic soils (2). Although the EqTS medium indice is obtained by the curves of responses as a function of the doses of P, the higher concentration of Ca in the DP compared to the TS may have provided greater amount of this nutrient to plants, which can have increased the efficiency of this by-product (DP = 18% Ca; TS = 14% Ca).

However, the EqTS medium was better than other indices such as Agronomic Efficiency Index (AEI), and equivalent

triple superphosphate (EqTS), because it did not suffer the influence of the dose used because it considers the part of the curves of greater response to the P addition.

The REE indices showed that studies about effects of P sources must consider the initial effect of the fertilizer but also the residual effect. The treatments without lime had the highest REE indices compared to those in which the corrective was applied.

The high REE achieved for DP is due to good agronomic efficiency indices associated with high PTS/PDP ratio = 1.90 (price of one kilogram of P_2O_5 of TS/price of one kilogram of P_2O_5 of DP). The results of this experiment for EqTS medium and REE suggest generally efficiencies of DP similar or above those of the TS, especially when not applied lime. At the same time, REE were not considered transportation and operation costs, which possibly were higher for DP due to be more the necessary quantity of this product, when compared to TS, to apply the same quantity of P.

On the other hand, these same results can direct field studies about the efficiency and use of DP in agriculture, assisting in the reduction of the national dependence on the import of phosphate fertilizers and the environmental liabilities of the fertilizer manufacturers.

The critical level values of P in soil falls within the range of P levels of the low soil fertility class (7 to 15 $mg\ dm^{-3}$) (9), which was established for annual crops, but is used for fodder species.

In the shoots of the plant, the critical level value is within the range suggested by some authors for *B. brizantha* (0.8 to 3.0 $g\ kg^{-1}$ of P) (15).

CONCLUSIONS

In the conditions of this experiment under greenhouse, it was concluded that the DP could be a good P source because it had high EqTS medium and REE, especially in acid soils and after the first growth period (residual effect).

The use of DP should consider that the liming reduces its agronomic and economic

efficiency. For improving the management of DP in agriculture, new studies in the field conditions should be performed.

Furthermore, the critical levels of P in the soil and shoots of the marandu grass were 15 mg dm^{-3} and 1.2 g kg^{-1} , respectively.

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Response of tomato (*Solanum lycopersicum* L.) to water consumption, leaf area and yield with respect to the number of stems in the greenhouse

Respuesta del tomate (*Solanum lycopersicum* L.) al consumo hídrico, área foliar y rendimiento con respecto al número de tallos en invernadero

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ABSTRACT

The objective of this study was to analyze tomato responses to water requirements (evaluated by means of balance lysimeters), leaf area, yield, quality and its relationship with weather, depending on the number of stems. The work was carried out in a greenhouse under hydroponic conditions. Tezontle (Stuff) was used as a substrate and a drip irrigation system was installed. The experiment consisted of three treatments, with one (T1), two (T2) and three (T3) stems per plant. The daily crop evapotranspiration was 0.30 L m⁻² in the initial stage, up to 4.41, 4.77 and 6.0 L m⁻², in the stage of maximum demand for T1, T2 and T3. The gross volume applied throughout the cycle was 352.2, 388.4 and 434.7 L m⁻² for T1, T2 and T3, with productivities of 49, 41 and 36 kg m³ and yields of 20, 18 and 16 kg m⁻² for T1, T2 and T3. Regarding quality parameters in size, T1 was the best, with 69, 23, 8 and 1% fruits of first, second, third and small fruits per plant respectively. The meteorological variables such as; temperature, wind, relative humidity, vapor pressure deficit and atmospheric water potential determined the consumption of water and nutrients in crops and are variables for irrigation scheduling.

Keywords

Solanum lycopersicum L. • balance lysimeter • tezontle • evapotranspiration • fruit quality

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RESUMEN

El objetivo de este estudio fue analizar las respuestas del jitomate a los requerimientos hídricos (evaluado por medio de lisímetros de balance), área foliar, rendimiento, calidad y su relación con el tiempo atmosférico, en función del número de tallos. El trabajo se realizó en un invernadero en condiciones de hidroponía. Se utilizó tezontle como sustrato y un sistema de riego por goteo. El experimento consistió en tres tratamientos, con uno (T1), dos (T2) y tres (T3) tallos por planta. El consumo por evapotranspiración diaria del cultivo fue 0,30 L m⁻² en la etapa inicial, hasta 4,41, 4,77 y 6,0 L m⁻² en la etapa de máxima demanda para T1, T2 y T3. El volumen bruto aplicado durante todo el ciclo fue 352,2; 388,4 y 434,7 L m⁻² para T1, T2 y T3, con productividades de 49, 41 y 36 kg m³ y rendimientos de 20, 18 y 16 kg m² para T1, T2 y T3. Con relación a los parámetros de calidad en tamaño, el T1 fue mejor; con 69, 23, 8 y 1% frutos de primera, segunda, tercera y frutos pequeños por planta. Las variables meteorológicas como temperatura, viento, humedad relativa, déficit de presión de vapor y potencial hídrico atmosférico determinan el consumo de agua y nutrimentos en los cultivos y son variables para calendarización del riego.

Palabras clave

Solanum lycopersicum L. • lisímetro de balance • tezontle • evapotranspiración • calidad de fruto

INTRODUCTION

Tomato crop (*Solanum lycopersicum* L.) is one of the most consumed vegetables in the world (Chapagain and Wiesman, 2004) and mainly grown under greenhouses. In Europe and the United States, the intensive production system in greenhouses has use undetermined growth habit cultivars and low densities that vary from two to three plants per square meter; where the stems of the plants are often pruned and set a single stem that reaches more than seven meters in length and it is left to harvest 15 or more bunches per plant in a single crop cycle per year (3). This production system in Mexico is relatively new and has generated an impact on the increase in cultivated area, productivity, profitability and quality in recent years.

Production under protected or forced conditions has several advantages over open field production: greater efficiency

in the use of water, soil and fertilizers, as well as greater flexibility in the sowing and harvest season according to the market demand.

By having better control over environmental and agronomic variables, greenhouse production is usually better, in quality and quantity than that produced at open field (7, 16, 18, 26).

Irrigation application timing and quantity determine the yield of crops in these systems. In addition, that helps to increase water use efficiency. Thus, a poor coupling of irrigation with water demands of the crop can promote the presence of pests, diseases and physiological disorders (1, 19, 25).

The necessary amount of irrigation should be applied to cover water consumption of the crop by the evapotranspiration (10).

Excess irrigation water generates leaching of soluble fertilizers which contributes to the pollution of ground-water. On the other hand, a lower supply to the crop evapotranspiration (ETc) can lead to water deficit and consequently a reduction in yield potential. Although there are several studies to determine irrigation requirements of open field tomato, its estimation in greenhouses is scattered and limited (28). Furthermore, studies under greenhouse conditions have been carried out for production systems in Europe, the United States and Canada, where there is usually a lower density of plants.

In Mexico, empirical equations have been used for determination of evapotranspiration in protected environments, but it is not known exactly if these values are correlated with the real evapotranspiration, since there is a lack of evapotranspiration data measured over the crop cycle. It is necessary to establish a simple and economical procedure in order to estimate the crop evapotranspiration in protected environments, that can allow adjust such estimation based on meteorological variables (29). The balance method is the most used method to directly estimate the evapotranspiration in which evaporation is measured from bare soil and crop transpiration. Lysimeters are containers with soil generally installed in the field under natural environmental conditions; where water, soil, plant, atmosphere system can be regulated, allowing to measure more accurately. Those lysimeters are used to study environmental effects and to evaluate the estimation methods for water demand of crops (8). That allows calculating the actual evapotranspiration of the crop (ETc) and thus estimating the loss of moisture in the soil, plant, and atmosphere system, which allows scheduling irrigation timing (13).

Greenhouse irrigation is usually applied on drip system with a pre-established interval and timing. This empirical practice does not consider the dynamic conditions in the management, environment and development of the crop. When there is no coupling between irrigation and crop evapotranspiration demands, there are periods of moisture deficit that affect the potential productivity of the crop and the efficient use of water and fertilizers (5).

Development of many organisms is controlled mainly by environmental temperature. Thermal time is an indirect measure of growth and development of plants and insects. These represent the integration of two temperature thresholds, which is define as interval in which an organism is active. Outside this interval, the organism does not show appreciable development or die.

The concept of thermal time resulted from observations indicated: 1) plants do not develop when the ambient temperature is lower than the basal one (Neild and Smith, 1997), 2) the rate of development increases when ambient temperature is higher than the basal one, 3) the varieties of tomatoes require different values of growing degree days.

Knowledge of tomato water demands will allow coupling this demand with the application of irrigation in a timely and efficient manner, and as a result, an increase in the efficiency of fertilizer application, a reduction in contamination, an increase in yield and fruit quality.

Objective

Analyze tomato responses to water requirements (evaluated by means of balance lysimeters), leaf area, yield, quality and its relationship with weather, depending on the number of stems.

MATERIALS AND METHODS

Description of the experiment

The experiment was carried out in a greenhouse at graduate college Montecillo Campus, State of Mexico, (19° 28'05" latitude North and 98° 54'31" longitude West at 2.244 m altitude). In a greenhouse with plastic covers of high-density polyethylene, 75% transmissivity, natural ventilation, and anti-aphid mesh screen equipment. The average temperature recorded during the crop growing cycle was 18.45 °C, 25.0 °C for the hottest month (April) and 9.0 °C for the coldest month (September).

Cultivar "*Cid F1*" seeds were sown on March 5th, transplanted on April 20th and harvested at the last bunches of fruits on September 20th, 2015. The plants were maintained at one, two and three stems, and were sprouted on July 8, 2015, when the tenth floral bunches were reached. The arrangement of transplanting consisted of separation of 40 cm between plants and 40 cm between lines in all treatments, using 35 x 35 cm black polyethylene bags with red tezontle (stuff) as substrate, with a crop density of 3 plants m⁻².

Treatments (T) consisted of three management conditions, depending on the number of stems per plant: with one (T1), two (T2) and three (T3) stems per plant, respectively. The area of each treatment was 53 m² with a total area of 159 m². The treatments were distributed in the field under a design in random blocks with arrangement in divided plots with four repetitions whose dimensions were 10 m². The drip irrigation system consisted in a surface irrigation line of 16 mm of diameter, with self-compensating dripper, flow rates of 4 L h⁻¹, an operating pressure of 68.64 k Pa. The nutrient solution was applied with Steiner solution (27).

The physical properties of five *tezontle* (volcanic rock) samples were determined. The results were as follows: water holding capacity 1.7 L, bulk density 1.04 g cm⁻³, total porosity 40.98%, air capacity 59.44%, field capacity 0.183 cm³ permanent wilting point 0.09 cm³ volumetric water content 0.092 cm³ cm⁻³.

Description of variables

Temperature (T_a, °C) and relative humidity (RH, %) were recorded with a data acquisition system, Hobo U12-011 was installed inside the greenhouse at 2 m above the ground. With these variables, the vapor pressure deficit (VPD, k Pa) and atmospheric water potential (Ψ_w) were computed from planting to harvest of the tenth bunches. The VPD is a useful variable to express the vapor flow (gradient) in a greenhouse. It also allows knowing the flow of the condensation and the crop transpiration. Besides, it quantifies the proximity of greenhouse air to the saturation.

To estimate the daily degree of development, it was used the (equation 1) that requires the average environmental temperature (17).

$$\begin{aligned} ADD &= T_a - T_{c-min} \text{ si } T_a < T_{c-max} \\ ADD &= T_{c-max} - T_{c-min} \text{ si } T_a \geq T_{c-max} \\ ADD &= 0, \text{ si } T_a \leq T_{c-min} \end{aligned} \quad (1)$$

where:

T_a = the daily air temperature
T_{c-max} and T_{c-min} = minimum and maximum air temperature, the threshold for the tomato growth are 29 and 11°C (24).

The accumulated values for n days are expressed with:

$$ADD = \sum_{i=1}^n GDi \quad (2)$$

Where:

i = the number of the day from the initial day, either at planting or the first phenological stage

To measure the evapotranspiration of the crop, control pots with 10 kg of tezontle and water holding capacity of 1.7 L. were used. In each treatment, four replicates of control pots were installed and covered with plastic on the surface to avoid evaporation of water from the substrate. In these pots, a known volume of water was applied at 9:00 a.m, which was allowed to drain for 50 minutes and the drained nutrient solution was measured, and by difference (volume applied and drainage), the evapotranspirated volume was obtained per plant (figure 1). This procedure was repeated daily at 11:00, 13:00, 15:00 and 17:00 hours with five daily measurements from transplant to harvest of the tenth bunches.

The evapotranspiration during a certain period was the difference between the water supply and the drainage:

$$ETc = R - D \pm \Delta\theta \quad (3)$$

where:

ETc = crop evapotranspiration

R = the irrigation volume (L)

D = the drainage volume (L)

$\Delta\theta$ = the rate of water content in the substrate (L)

In the absence of rain and other inputs, the irrigation requirements are equal to actual crop evapotranspiration (ETr) described by the following equation.

$$RR = ETr = \text{transpiration (L)} \quad (4)$$

In addition, pH and EC in the nutrient solution supplied and drained were measured from the pot in order to know the concentration of ions in the solution. In order to adjust the fertilization scheduling to carry out a better control in supplying the nutrient solution.

Relationship between leaf area index and water requirement

In addition, from the measurement of water consumption, the leaf area index was measured in different crop stages to correlate with the water demand of the plant.

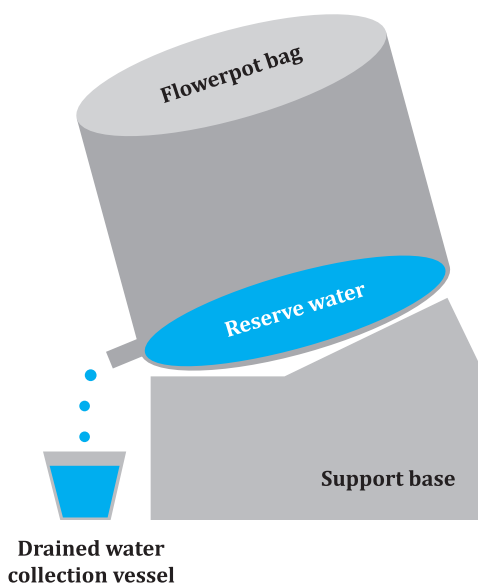


Figure 1. Daily evapotranspiration measurement in control pots in the greenhouse.

Figura 1. Medición de evapotranspiración diaria en macetas control en invernadero.

In each harvest, quality classification of fruit size was divided into five categories (extra-large, large, medium, small and smallest fruits) based on the Mexican Standard (table 1).

To determine the significant differences of the evaluated variables, analysis of variance (ANOVA) was conducted along with Tukey mean separation test ($P \leq 0.05$) using MINITAB statistical software.

Water use efficiency (WUE) estimated as the ratio of total yield (kg) and the volume of water applied (m^3) as well as yield (R) of the treatments mass produced per unit surface ($kg\ m^{-2}$).

Electric conductivity and pH values managed

The pH values of the nutrient solution were the average of 6.85, and ranged from 6.7 to 7.0; for electrical conductivity (C.E) the average was $1.85\ dS\ m^{-1}$ and varied between 1.1 and $2.6\ dS\ m^{-1}$. The average values for drained solution were 7.6 for pH and $2.89\ dS\ m^{-1}$ for electrical conductivity. These values of electrical conductivity for the drained solution were lower, that recommended for production in greenhouses, whose typical interval is between 2 and $4\ dS\ m^{-1}$.

RESULTS AND DISCUSSIONS

Temperature and relative humidity

At the initial stage of crop, the temperature reported was higher than $22^\circ C$, during the hottest months from April to June, and then decreased. In the rainy months from July to September, it decreased until $9^\circ C$, and increased the relative humidity up to 90%, due to the rain, cloudy days at the beginning of the cold season where the experiment took

place (figure 2, page 93). The behavior of these two variables were similar to those reported by Jaimez *et al.* (2005).

Vapor Pressure Deficit (VPD)

Vapor Pressure Deficit (VPD) is an important variable in the crop production, either, in protected conditions or open field. It indicates that when air becomes saturated, the water will condense to form clouds, dew or water films over the leaves. This is the last instance that makes the VPD important for the regulation of water demand. If a film of water keeps on the leaf of the plant, it becomes much more susceptible to putrefaction (20).

The VPD in the stage of vegetative development was $1.7\ k\ Pa$. This value could be due to the initial phase of growth, its leaf area was small, so the number of stomata was scarce.

Although the transpiration rate was high, as well as the loss of water vapor inside the greenhouse, this did not reach to satisfy the atmospheric demand. This process involved greater release of water vapor through the stomata of the leaves trying to cover the atmospheric water demand (figure 3, page 94).

Table 1. Saladette tomato fruits classification (15).

Tabla 1. Clasificación de frutos de jitomate tipo saladette (15).

Size	Diameter [mm]	
	Minimum	Maximum
Smallest fruits	26	37
Small	37	51
Medium	51	60
Large	60	71
Extra-large	71	Onwards

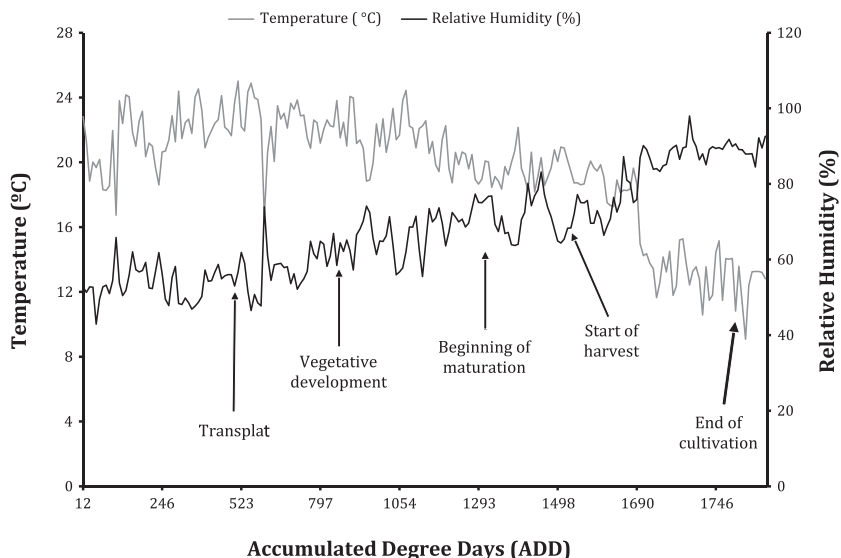


Figure 2. Temperature and relative humidity in the greenhouse during the crop cycle.
Figura 2. Temperatura y humedad relativa en el invernadero durante todo el ciclo de cultivo.

A high VPD increases the transpiration demand, increases water and nutrient uptake, increasing the photosynthetic activity, in addition, it directly influences the amount of water in plant tissues that is further transferred to the greenhouse air. In the final stage of cultivation, the VPD close to 0.1 kPa was estimated, since the plants decreased the transpiration, because the atmosphere was practically saturated with vapor water.

Therefore, less diffusion of the water vapor from the stomata to the environment was present which reduced the photosynthetic activity, which had an impact on yield and quality of the fruits (figure 3, page 94).

In addition, a very low VPD indicated near to the dew point could be harmful for the crop. These results were reported by Jaimez *et al.* (2005) with 0.4 kPa minimum

and 2.5 kPa maximum of VPD of red pepper cultivated in controlled conditions.

The behavior of VPD during a day (24 hours), each minute was completely different from the daily VPD. In the vegetative stage, the lowest value of VPD was found of 0.16 kPa at 6:23 a.m. and the highest value of 10.76 kPa at 13:30 p.m., corresponded at 20 days after planting (six true leaves). These compared to the VPD obtained in the reproductive stage, the lowest value of 0.11 kPa was presented at 7:31 a.m. and during the day of solar insolation at exactly 15:07 p.m. the highest value of 5.27 kPa was estimated, corresponding at 131 days after planting (ripening of fruits of the 1st bunch).

The high peaks did not occur at the same time; this was due to the variation of the season of the year.

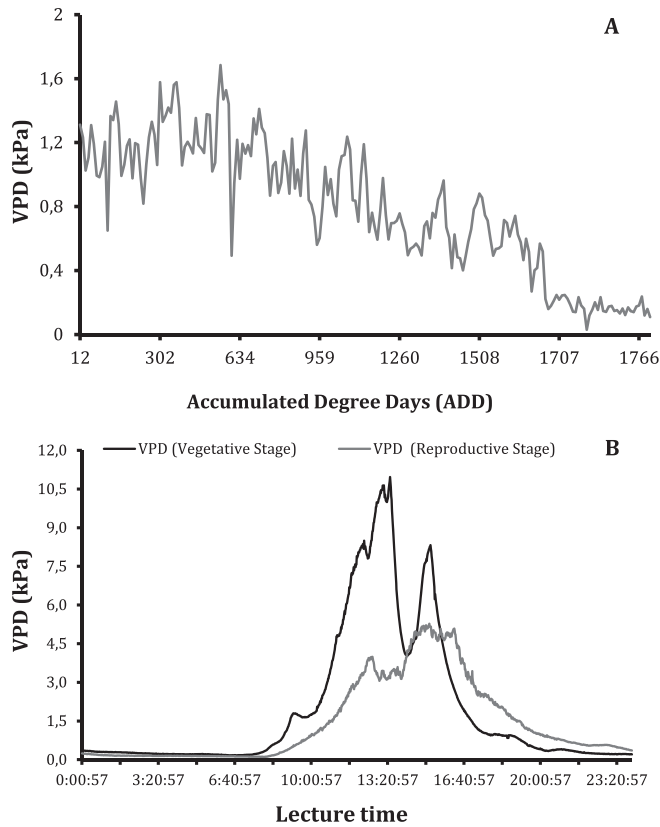


Figure 3. Daily vapor pressure deficit (A) and for a day (B) in the greenhouse.
Figura 3. Déficit de presión de vapor diario (A) y durante un día (B) en invernadero.

Although the values were out of the optimum range, the crop was developed in a normal manner and did not present problems of pests or diseases, this was attributed to an adequate management in the pruning of shoots and preventive application of fungicides and insecticides during the crop cycle (figure 3).

As the VPD increases, the plant needs to extract more water from the substrate through its roots to satisfy the water and nutrient demand. For this reason, the adequate values for the VPD in the greenhouse are from 0.45 kPa to 1.25 kPa, the

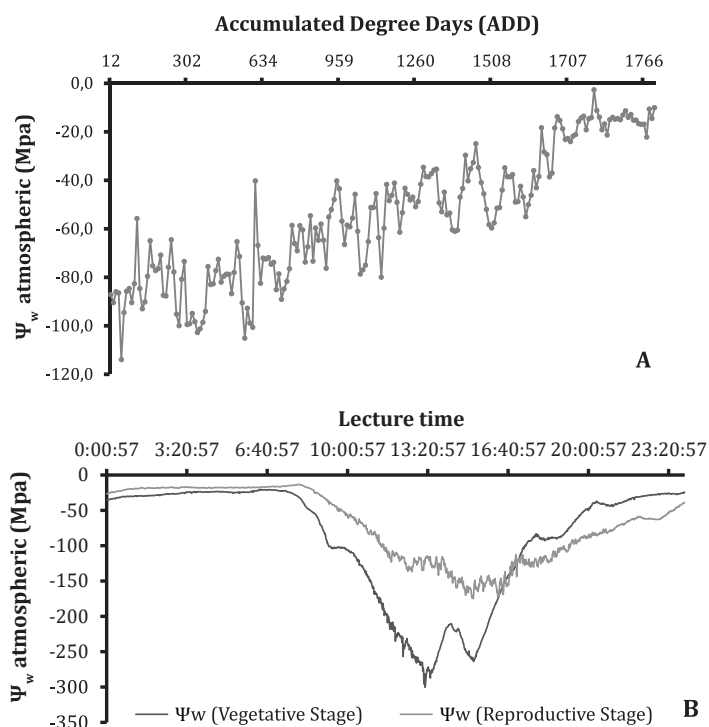
optimum are around 0.85 kPa. Overall, most plants have an optimal VPD between 0.8 and 0.95 kPa (21). With the calculation of current VPD, it is known the susceptibility of the crop to develop diseases due to environmental conditions or to susceptibility of a pest development. Prenger and Ling (2001) showed that fungal pathogens survive better below VPD (<0.43 kPa).

In addition, infection of the disease is more detrimental below (0.20 kPa). Therefore, the climate of the greenhouse should be maintained above (0.20 kPa), to prevent diseases and damage to crops.

Atmospheric water potential (Ψ_w)

Atmospheric demand is undoubtedly a factor of great importance to determine the amount of water that a crop requires for its growth and development. This demand will depend on the incident radiation, temperature, relative humidity, and wind. Figure 4 shows the atmospheric water potential (Ψ_w) at higher temperature in the vegetative stage lowers the atmospheric water potential which means that the atmosphere requires more water to saturate the environment, which can

be evidenced by the dropped down of Ψ_w of the dry air. At the end of the crop cycle, there was an increase in Ψ_w due to the air temperature dropped down and the relative humidity raised, which corresponded to the start of the rainy months, cloudy days. With relative humidity of 100% at any temperature, the water potential of the air was equal to zero (figure 4). As the atmospheric demand increased, the evapotranspiration crop increased too the water and nutrients uptake to a certain threshold, fixed by the potential of water from its leaves.



Figures 4. Daily atmospheric water potential (A) and during a day (24 hours) each minutes (B) in greenhouse.

Figura 4. Potencial hídrico atmosférico diario (A) y durante un día (24 horas) en intervalo de cada minuto (B) en invernadero.

López (2000) reported that when the relative humidity (RH) of the air was 98% at 20°C, the water potential of the air decreased to -2.72 MPa, which was enough to raise a water column at a height of 277 m. At 90% of RH, the $\Psi_w = -14.2$ MPa; at 50% RH of air, $\Psi_w = -93.5$ MPa and at 10% RH, $\Psi_w = -311$ MPa. The water potential of the soil water available to the plants was rarely below -1.5 MPa, was not needed to generate a pronounced water potential gradient from the soil passing through the plant to the atmosphere due to the dry air. Even when the soil is very humid and the RH of the air is 99%, a gradient of water potential can be established.

In addition, Gil-Pelegrín, *et al.* (2005) found similar behavior of atmospheric hydric potential through a continuous integrating model soil-plant-atmosphere (SPAC) that analyzes the flow of water in terrestrial plants as a dynamic process along a series of compartments, from the source (soil) to the final demand (atmosphere).

The behavior of Ψ_w atmospheric during a day (24 hours), each minute was completely different from the daily Ψ_w . In the vegetative stage, the lowest value of Ψ_w was found of -20.05 MPa at 6:23 a.m. and the highest value of -300.03 MPa at 13:13 p.m., corresponded at 20 days after planting (six true leaves). These compared to the Ψ_w obtained in the reproductive stage, the lowest value of -13.26 MPa was presented at 7:58 a.m. and during the day of solar insolation at exactly 15:09 p.m. the highest value of -173.60 MPa was estimated, corresponding at 131 days after planting (ripening of fruits of the 1st bunch).

Crop evapotranspiration

The daily evapotranspiration variation per m², measured with the balance method, is shown in figure 5a, 5b y 5c, page 97. For the treatment of one stem (T1), it was

observed that in the initial phase the daily variation of water demand was between 0.3 - 4.41 L m², in the stage of maximum water demand (at the maturation stage of fruits of the 1st bunch) at 1500 ADD (figure 5A, page 97).

In addition, it was found that the plant began to develop its leaf area, height, roots and fruits, the water requirement increased. These data were closer to reported by Flores *et al.* (2007) for tomato crop in greenhouse with a crop density of 4.3 plants m⁻² in tezontle.

For the treatment of two stems (T2) the daily variation of the water demand was between 0.3-4.77 L m⁻² plants⁻¹ from the initial phase to the stage of maturation of fruits (the 1st bunch) to the 1500 DDD (figure 5B, page 97). The volume of water uptake by this treatment was closer to (T1) in the vegetative stage, however, at the beginning of maturation, differences were found by increasing the volume of water uptake.

The water requirement for three stems (T3), the daily variation was from 0.3 to 6.0 L m⁻² from the initial stage to the stage of ripening of fruits (the 1st bunch) to the 1500 DDD (figure 5C, page 97). These results were closer to those reported by León *et al.* (2005) for Lignon and FL-5 varieties of tomato grown under protected conditions on two sowing dates.

The volume of water uptake in the three treatments at the beginning of the crop cycle was minimum because leaves and roots were not fully developed in that they did not consume a greater volume of water. After 750 DDD, the volume of water applied to the crop increased, because the development of the plant and its total biomass was increased too as a function of time. The changes of the variations of water requirement in the three treatments were due to the effect of cloudy days, rainy days and clear days.

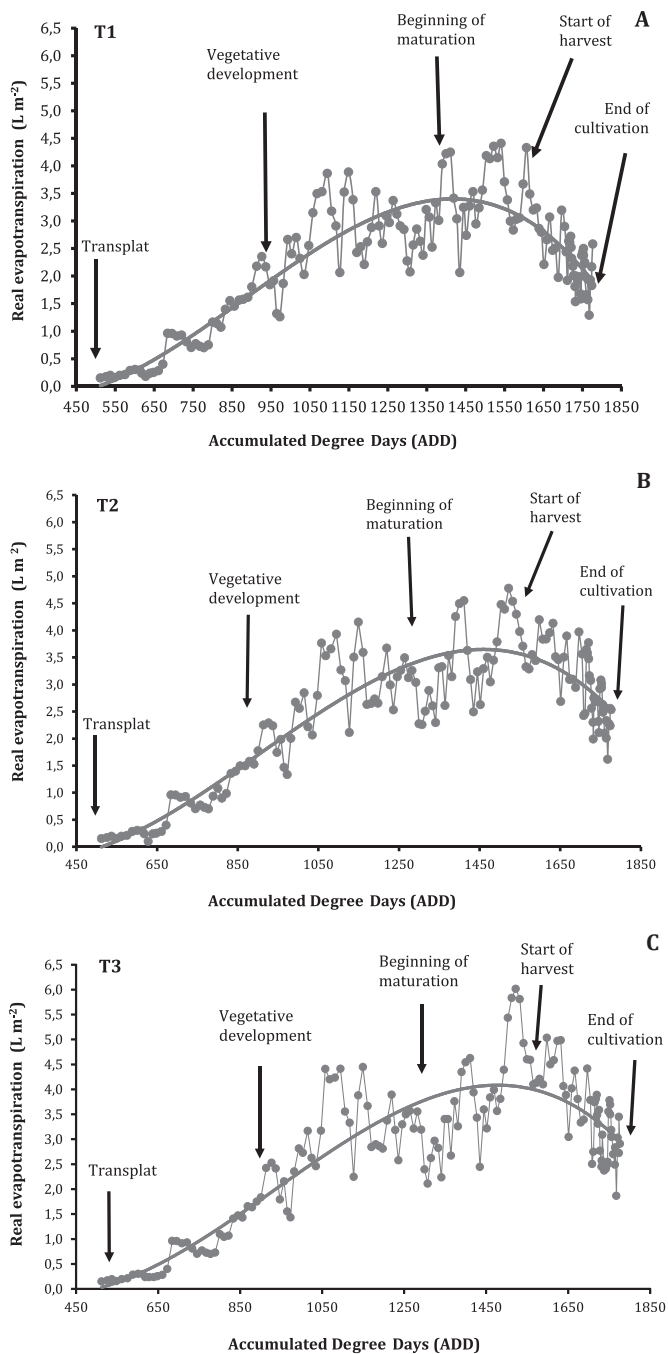


Figure 5. Net water requirements for T1, T2 and T3.
Figura 5. Requerimiento hídrico neto para T1, T2 y T3.

Net water requirement each crop stages for one stem

The net consumption measured by the balance method during the whole crop cycle was 352.2, 388.4 and 434.7 L m⁻² for T1, T2 and T3 respectively, in a cycle of 154 days, from the transplant to the tenth bunches (table 2, 3 and 4, page 99).

According to the results obtained from Tukey mean separation test (95%), no significant difference in the average of net water requirement was found in the three replications among the tomato cultivation treatments grown in the greenhouse (table 5, page 99).

Relationship between leaf area index and water requirement

The values of water uptake obtained during the growing cycle, without water limitations in the area explored by the roots, depended on the atmospheric demand, the duration of the growing season and the foliar area developed by the plant. In terms of crop water consumption, the aerial structure of the crop determined the transpiration crop. When the leaf area index increased, the water consumption of the crop rised linearly to the atmospheric demand, reaching a maximum value under maximum growth of the canopy, declining with the progression of the season (figure 6, page 100).

Water yield and productivity

The yield of the crop pruned to one stem (T1) was 20 kg of fruit per m⁻², this yield was similar reported by Flores *et al.* (2007) for tomato c.v. Tequila, in addition, Corella *et al.* (2013) obtained yield of 23.43 kg m⁻² for tomato c.v. Malinche cultivated on a single stem in the greenhouse.

The yield of the crop pruned to two stems (T2) was 18 kg m⁻², this yield was found by Corella *et al.* (2013) of 18.55 and 18.24 kg m⁻² for c.v. 4426 and c.v. Aníbal,

respectively, for indeterminate tomato crop growth in greenhouse.

The yield of the crop pruned to three stems (T3) was 16 kg m⁻². Information was not found for the yield and quality for this last treatment. Significant statistical differences were found between treatments. The gross volume applied throughout the growing cycle was 404, 438 and 444 L m⁻² for T1, T2 and T3 respectively, with a productivity of 49, 41 and 36 kg m⁻³ of water, Flores *et al.* (2007) reported 35 kg m⁻³ for tomato c.v. Tequila of indeterminate growth. This indicates that higher evapotranspiration does not necessarily indicate higher yield.

Size of fruits by category

Regarding the size of the tomato fruits in (%) harvested for each treatment. For one stem (T1) it was better with 69, 23, 8 and 1% fruits of large, medium, small and smallest fruits, respectively (table 6, page 102). The fruits obtained in this treatment comply with the NMX-FF-031-1997 to be exported to the international market. The results obtained in this work were found by Rodríguez *et al.* (2008), for tomato under greenhouse conditions, found that 60% corresponds to extra quality; 20% at first, 10% at second and 10% at loss.

For two stems (T2) 49, 33, 17 and 1% fruits of large, medium, small and smallest fruits were obtained respectively. The fruits obtained in this treatment could be considered for the national market due to its size, it is mainly transferred to supermarkets and central supply stores for its later distribution to final consumers. Quintana *et al.* (2010) reported the results, for tomato in greenhouse, they reported that 9% corresponds to extra quality, 52% to first, 27% to second, 11% to third and 2% to fourth.

Table 2. Net water requirement each crop stages for one stem (T1) of tomato.

Tabla 2. Requerimiento hídrico neto por etapa de cultivo de jitomate para un tallo (T1).

Stage	Water consumption			Days after planting		Degrees-day development
	Daily	Period	Accumulated	Period	Accumulated	Accumulated
	L m ⁻² d ⁻¹	L m ⁻²				
Initial	0.30	4.80	4.80	16	16	670
Vegetative	1.35	33.75	38.55	25	41	951
Development	2.85	88.35	126.90	31	72	1268
Production	3.12	165.36	292.26	53	125	1698
End of cycle	2.07	60.03	352.29	29	154	1762
CV(%)	1.15	61.43	153.59	13.68	57.38	470.91

Table 3. Net water requirement each crop stages for two stem (T2) of tomato.

Tabla 3. Requerimiento hídrico neto por etapa de cultivo de jitomate para dos tallos (T2).

Stage	Water consumption			Days after planting		Degrees-day development
	Daily	Period	Accumulated	Daily		Period
	L m ⁻² d ⁻¹	L m ⁻²				
Initial	0.30	4.80	4.80	16	16	670
Vegetative	1.35	33.75	38.55	25	41	951
Development	2.97	92.07	130.62	31	72	1268
Production	3.42	181.26	311.88	53	125	1698
End of cycle	2.64	76.56	388.44	29	154	1762
CV(%)	1.28	67.41	168.71	13.68	57.38	47.91

Table 4. Net water requirement each crop stages for three stem (T3) of tomato.

Tabla 4. Requerimiento hídrico neto por etapa de cultivo de jitomate para tres tallos (T3).

Stage	Water consumption			Days after planting		Degrees-day development
	Daily	Period	Accumulated	Daily		Period
	L m ⁻² d ⁻¹	L m ⁻²				
Initial	0.30	4.80	4.80	16	16	670
Vegetative	1.38	34.50	39.3	25	41	951
Development	3.24	100.44	139.74	31	72	1268
Production	3.87	205.11	344.85	53	125	1698
End of cycle	3.03	87.87	432.72	29	154	1762
CV(%)	1.48	76.88	188.68	13.68	57.38	470.91

Table 5. Comparison test of means of average net water requirement between treatments.

Tabla 5. Prueba de comparación de medias de requerimiento hídrico neto promedio entre los tratamientos.

Treatments	Initial stage	Vegetative stage	Development stage	Production stage	Final break
One stem (T1)	0.30 a	1.35 a	2.85 a	3.12 a	2.07 a
Two stems (T2)	0.30 a	1.35 a	2.97 a	3.42 a	2.69 a
Three stems (T3)	0.30 a	1.38 a	3.24 a	3.87 a	3.03 a
CV (%)	0.0	0.0	0.2	0.4	0.5

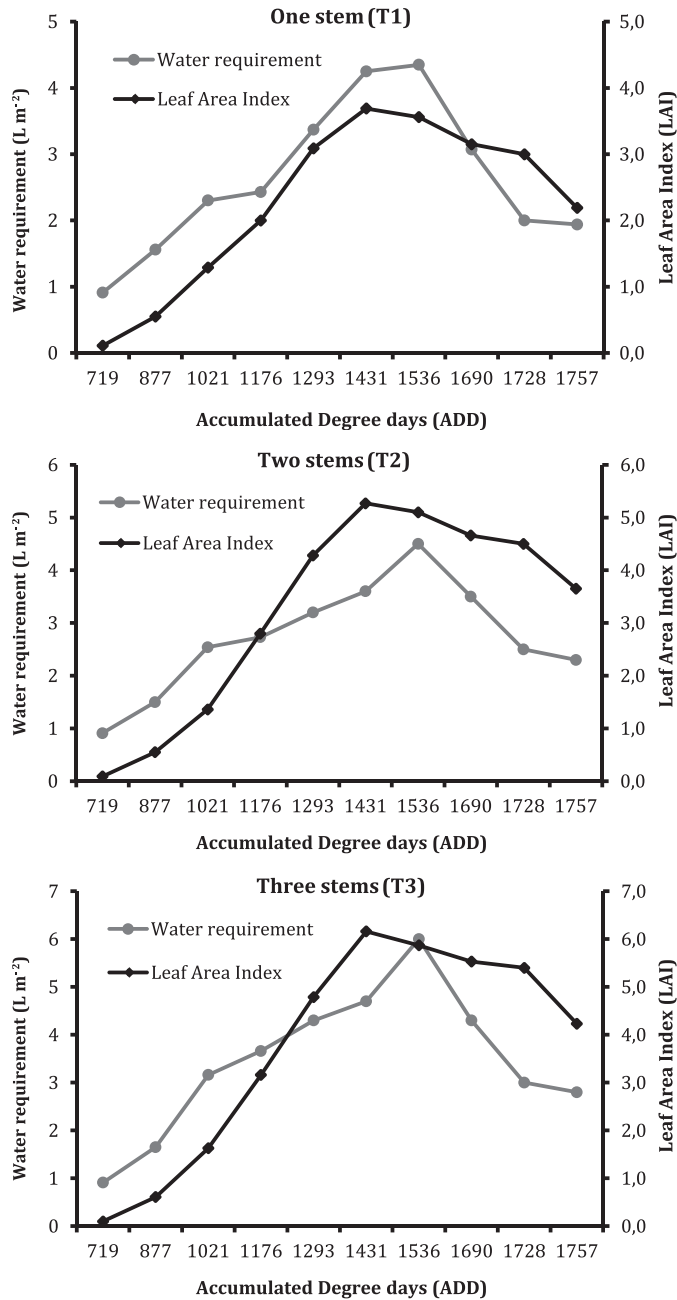


Figure 6. Ratio of water requirement and leaf area index during the whole crop cycle for T1, T2 and T3.

Figura 6. Relación de requerimiento hídrico e índice de área foliar durante todo el ciclo de cultivo para T1, T2 y T3.

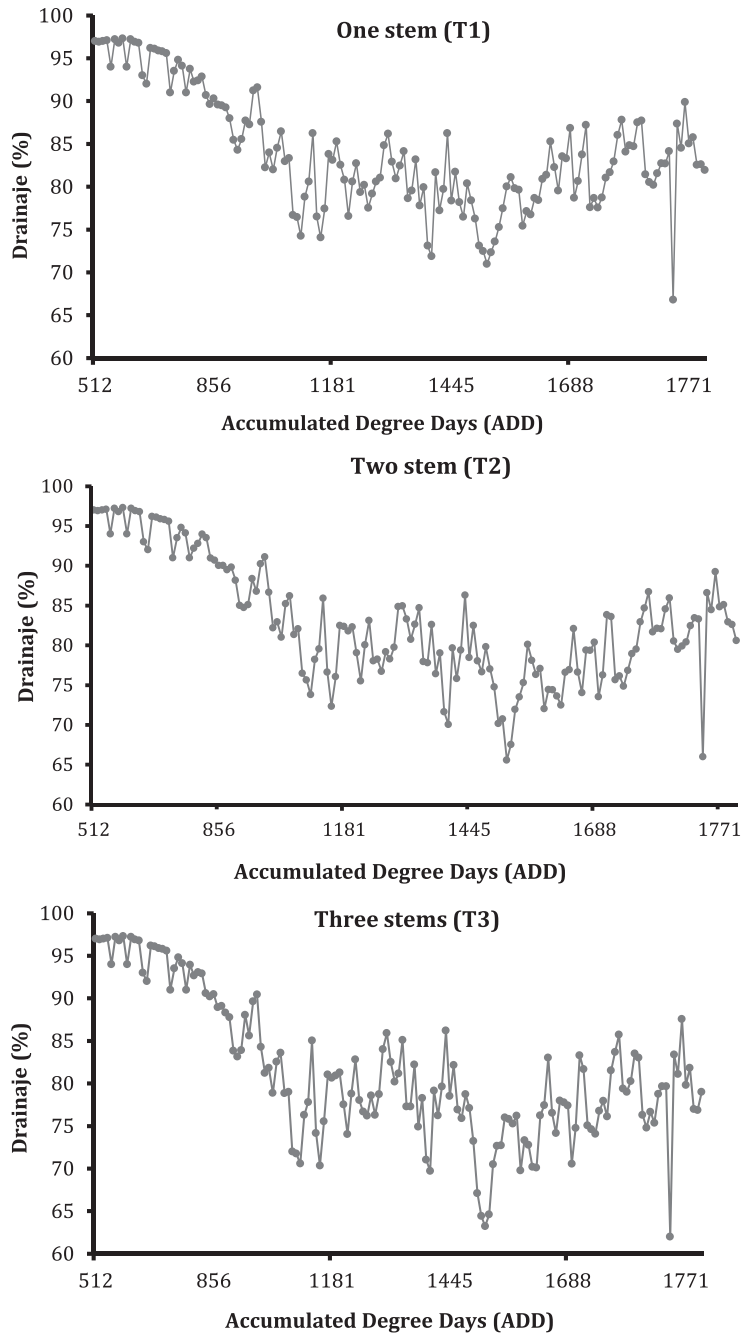


Figure 7. Daily drained volume variation for T1, T2 and T3.
Figura 7. Variación del volumen drenado diario para T1, T2 y T3.

Table 6. Quality classification of the size tomatoes fruit.**Tabla 6.** Clasificación de calidad en tamaño de los frutos de jitomate.

Treatments	Large (%)	Medium (%)	Small (%)	Smallest fruits (%)
One stem (T1)	68.70 a	22.93 c	7.66 c	1.66 b
Two stems (T2)	49.10 b	33.17 b	16.66 b	1.05 ab
Three stems (T3)	36.77 c	38.54 a	23.00 a	0.68 b
CV (%)	16.1	7.9	7.7	0.5

For three stems (T3), 37, 39, 23 and 2% of fruits of large, medium, small and small fruits were obtained, respectively. The fruits obtained in this treatment can be considered for the local market since the majorities are fruits are of medium size. These fruits are consumed in fresh condition, and are used in the preparation of puree or sauce for food.

Least significant difference: means followed by the same letter within a column are not significantly different according to the Tukey test ($\alpha = 0.05$).

Drainage measured in the lysimeter

A leaching irrigation was applied in the control pots to ensure the drainage volume. The crop was kept under optimum humidity conditions.

In figure 7 (page 101) presents the daily variation of the fraction drained in the lysimeter, with respect to the applied volume.

The drained volume in the initial stage in the three treatments was 97%, this was due to the newly transplanted plants where the volume of water consumption was minimal and in the stage of maximum demand (ripening of the bunch) the volume drained was 70, 65 and 61 % for T1, T2 and T3 respectively. In this stage, the volume of drainage decreased due to the increasing in development and fructification of the plant over time, thus it raised the water demand because of the filling of the fruit. These drainage variations

were found by Flores *et al.* (2007) in tomato cultivation of 0-80%. For soils or substrate with fine texture it is recommended that the percentage of drainage does not exceed more than 30%. Since it can decrease oxygen content in soil and roots showing damages due to lack of respiration and presence of fermentation and this it will be reflected in the decrease in quantity and quality of fruits.

CONCLUSIONS

The results indicated that it is possible to estimate irrigation requirements in greenhouse crops by means of balance method. These lysimeters (drainage) are economical, easy to install and measure the water uptake, with the condition that should be there drainage and, there must be strict control in the application of irrigation.

With proper handling it helps us to determine the volume of irrigation. The experimental values indicated that the tomato irrigation requirements vary from 0.30 L m⁻² for the three treatments in the initial stage, up to 4.41, 4.77 and 6.0 L m⁻² in the stage of maximum demand for T1, T2 and T3, respectively. The maximum value of the irrigation requirement was lower than that reported in the literature for low planting densities, which indicated the importance of estimating the irrigation requirements of crops for local conditions.

The gross volume of water applied during the crop cycle was 404, 438 and 444 L m⁻² for T1, T2 and T3, respectively. With a productivity of 49, 41 and 36 kg of fruit per m⁻³ of water and a yield of 20, 18 and 16 kg of fruit per m⁻², which indicated the importance of generating optimum foliage to obtain maximum yield. The best treatment was T2 (two stems) since the fruits obtained were more homogenous.

A better knowledge of the daily water demands of the crop facilitates adjusting the volume required for irrigation, depending on the phenological stage of the crop and the weather conditions coupling to the concept of degree-day development, as a function of temperature. In addition, the pruning of tomato crop is a cultural practice used to obtain vigorous plants, resulting in fruits of better quality and higher yields (2).

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Solarized manure and two irrigation methods on fruit yield of jalapeno chili (*Capsicum annuum* L. var. *annuum*)

Estiércol solarizado y dos métodos de riego en la producción de chile jalapeño (*Capsicum annuum* L. var. *annuum*)

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ABSTRACT

Increase of water use efficiency in crops through better irrigation systems and use of farmyard manure to fertilize plants and avoid contamination are needs in arid regions. The objective was to evaluate a jalapeño pepper crop with organic fertilization and two irrigation methods. This research was carried out at Gomez Palacio, Durango, Mexico, in 2013 and 2014. The experimental units were randomly distributed in a split plot design with four replications. Main plots were irrigation treatments and fertilizers were applied as split plot treatments within each irrigation treatment. Irrigation was applied with either a furrow irrigation system to apply 80% of the measured pan evaporation or drip irrigation set to deliver 60% of the measured evaporation. The drip irrigation treatment showed greater fruit yield than the surface irrigation ($p < 0.05$). Green fruit yield was similar among fertilized treatments in 2013 ($p > 0.05$) and different in 2014 ($p < 0.05$) with 40 t ha⁻¹ solarized manure having the greatest fruit yield. Soil organic matter and nitrate-N content were greatest in 2013 when solarized manure 40 t ha⁻¹ was used. There were no differences among fertilizer treatments in 2014 ($p > 0.05$). Soil pH and electrical conductivity remained unchanged among treatments in each year of the study. Drip irrigation had a positive effect in the fruit yield in spite of delivering a lesser amount of water. The solarized manure applied at 40 t ha⁻¹ had the greater effect in the fruit yield in year 2 but not in year 1.

Keywords

fruit yield • fertilizers • nitrates • organic matter • evaporation

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RESUMEN

Incrementar el uso eficiente del agua a través de mejores sistemas de irrigación y el uso de estiércol de vaca para fertilizar plantas y evitar la contaminación son necesidades en regiones áridas. El objetivo fue evaluar un cultivo de chile jalapeño con fertilización orgánica y dos métodos de irrigación. La investigación fue llevada a cabo en Gómez Palacio, Durango, México en 2013 y 2014. Las unidades experimentales fueron distribuidas aleatoriamente en un diseño de parcelas divididas con cuatro repeticiones. Las parcelas grandes fueron los tratamientos de irrigación y los tratamientos de fertilización fueron las subparcelas. El agua aplicada a través de surcos de irrigación fue el 80% de evaporación medida en un tanque evaporímetro o bien a través del riego por goteo para aplicar 60% del agua evaporada. El tratamiento de riego por goteo mostró mayor cosecha de fruto que el riego por surcos ($p < 0,05$). La cosecha de fruto fue igual entre los tratamientos de fertilización en 2013 ($p > 0,05$) y diferente en 2014 ($p < 0,05$) con el tratamiento de 40 t ha^{-1} de estiércol solarizado teniendo la mayor producción de fruto. La materia orgánica del suelo y el contenido de nitratos fueron mayores en 2013 en el tratamiento 40 t ha^{-1} de estiércol solarizado. No hubo diferencias entre tratamientos de fertilización en 2014. El pH del suelo y la conductividad eléctrica no tuvieron cambios entre tratamientos en los dos años de estudio. El riego por goteo tuvo un efecto positivo en la producción de fruto a pesar de que se aplicó menos cantidad de agua. El estiércol solarizado aplicado a 40 t ha^{-1} tuvo el mayor efecto en el año 2 pero no en el año 1.

Palabras clave

producción de fruto • fertilizantes • nitratos • materia orgánica • evaporación

INTRODUCTION

In Mexico there are approximately 500,000 ha in irrigation districts, mainly in arid and semiarid areas where water problems are exacerbated by the extraction rates exceeding infiltration rates which it leads to salinization; in regions such as the Laguna region, which is located to the northeast part of the State of Durango and in the southwestern State of Coahuila (22). This region extracts an average of 930.92 million m^3 per year but only 518.9 million m^3 are recharged. Therefore the ground water table loses almost two meters per year (9). Surface irrigation accounts for 94% of the irrigated land area in Mexico. In the La Laguna region low efficiency in irrigation systems

account for 63% of the water losses which includes water loss from storage dams to crop areas (9). There is, therefore, the need to increase irrigation efficiency by changing to pressurized systems. Water use efficiency can also be further increased by improving fertilization techniques which should provide more biomass per m^3 of water. Implementation of suitable irrigation methods that apply lower amounts of water, especially during the germination stage when it is difficult to keep the seedbed sufficiently moist, would improve crop yield. Economic crops such as jalapeno pepper would benefit the region with improved water use efficiency (4, 28).

Another problem in the Laguna Region is an unmanaged use of dairy manure.

The region is home of a burgeoning dairy industry of over 400,000 head that contributes over 900,000 t of dry matter per year (14). The manure is not properly utilized for crop production but with proper management it could provide organic nutrients for plants as it decomposes in the soil. The current practices include direct application of manure to agricultural soils without any regard to an optimum rate of application.

Manure appears to be over-applied but with no available studies in the region to determine an optimum rate for a given crop it is not known what side effects may be occurring (15). Manure could also be composted or vermi-composted if it were known what effects it would have on crop growth.

The use of manure or compost is becoming increasingly important and efficient means of recycling of nutrients, which helps crop growth and returns many of the elements extracted during the milk production process to the ground (31).

Peppers are an important commodity around the world and Mexico is the second largest producer in the world with an area of 143,465 ha, producing 2.73 million t (13). The jalapeno pepper (*Capsicum annuum* L. var. *annuum*) is one of the most important economic chiles within those grown in Mexico for its wide consumption, high profitability and a high labor demand (31). In response to these problems this research was conducted in jalapeno pepper where the objectives were to find optimum amounts of solarized cow-manure for jalapeno, and determine if drip irrigation could improve water use efficiency over that of furrow irrigation.

MATERIALS AND METHODS

This study was carried out in 2013 and 2014, in the experimental agricultural field (CAE) of the Facultad de Agricultura y Zootecnia (FAZ) de la Universidad Juárez del Estado de Durango (UJED), located at km 30 of the Gomez Palacio-Tlahualilo road in ejido Venecia, municipality of Gomez Palacio, Durango, Mexico (25°46'56" N and 103°21'02" W, 1110 m a. s. l.).

The climate, according to the classification of Köppen, modified by García (2004), is steppe (BS) and desert (BW), an arid climate with an average annual rainfall of 230 mm, a yearly evaporation of 1380-2530 mm, and an average annual temperature of 20.7°C. Rainfall during the study was 79.9 mm in 2013 and 153.4 mm in 2014, maximum average temperature was 33.4°C and 33.5°C, and minimum average temperature 20.0°C and 20.5°C in 2013 and 2014.

Solarization of manure

Manure was solarized by making a mound 1.0 m high and 2.0 m wide and 3.0 m long, after that it was covered with a polyethylene plastic without albedo which was held tight by applying soil around the edges of the plastic. Water was applied at the beginning of solarization to reach a moisture content of 55% in order to have better heat distribution through the manure. Solarized manure was analyzed using the Kjeldahl method for total N (21) finding 1.42%, at the time of incorporation into the soil in the laboratory of the National Center of Disciplinary Research in Water-Relationship Floor-Plant-Atmosphere (CENID-RASPA) of Gomez Palacio Durango, Mexico.

Treatments

The treatments resulted from the combination of two factors under a split-plot design randomly distributed. The whole plot was the irrigation methods with 4 repetitions (drip and furrow irrigation); the split-plot fertilization treatments of 20 and 40 t ha⁻¹ of solarized manure, a chemical treatment of 160-80-00 (NPK kg) and zero fertilization with 8 repetitions. All plots were first prepared by plowing to a depth of 30 cm followed by harrowing and land leveling. Experimental units (EU) were 3.2 m wide x 3 m long, with 8 EU having each fertilized treatment including the control treatment. The research was carried out at the same place, with the same treatments administered to the experimental units.

Dairy manure was exposed to solar radiation for two months before it was applied to the soil. Manure was incorporated into the soil in the first year by using a scraper to get a uniform area on the ground before plowing. In the second year manure was incorporated by harrowing. Synthetic fertilizer (80-80-00) was applied individually at transplanting as 153.8 kg ha⁻¹ of monoammonium phosphate (11-52-00) and 137.13 kg ha⁻¹ of urea (46-00-00).

The remainder nitrogen (80-00-00) was applied at 50 days after transplanting as 173.91 kg ha⁻¹ of urea. Drip irrigated plots were watered every four days corresponding to 60% of the amount of pan evaporation that had occurred for a total of 60 cm in 2013 and 51 cm in 2014. The irrigation system consisted of PVC pipe (0.019 m ID) where there were connected 20 cm length polyethylene hoses (0.013 m ID) each 0.8 m.

The other side of each polyethylene hose was connected to a drip tape (0.016 m ID, wall thickness 0.152 mm) with emitters

spaced every 0.15m with a hydraulic output (Q) of 0.65 L/h at 1.0 bar pressure.

Furrow irrigation was every 15 days. A total of 78 cm of water was applied in 2013 according to 80% of pan evaporation (Type A tank EV). There was 63 cm applied in 2014. Jalapeno seedlings var. 'Compadre' were transplanted on April 23, 2013, and May 05, 2014, transplants had 10 true leaves and a height of 20 cm, including the roots. Transplants were first seeded 50 days before transplanting. Distance between plants was 0.4 m and between rows 0.8 m, which gives a density of 31250 plants ha⁻¹.

The amount of nitrogen in manure treatments was based on analysis of the cattle manure, 1.42%, and considering that 30% of the N in the manure would be mineralized in the first year (11, 12).

Before planting in 2013, 10 random soil samples were taken from the entire treatment location. Soils were sampled again at the end of the last harvest in each year by taking one sample in each experimental unit. One kg of soil was collected from each of two depths (0-30cm depth and 30-60cm). Soil parameters that were measured in the experimental site were organic matter, soil texture, nitrate-N, pH and electrical conductivity. Amount of organic matter was estimated with the Walkley-Black method and soil texture with the Bouyucos method (26). The nitrate concentration was estimated with a transnitration of salicylic acid (23), electrical conductivity and pH by using an electrical spectrophotometer (Genesys 10S UV-Vis) (23).

Before planting, soil organic matter values were 2.41 and 1.46%, nitrates 3.80 and 1.12 mg kg⁻¹, electrical conductivity 3.13 and 3.55 dS m⁻¹, pH 7.8 and 7.06 at the soil depth of 0-30 cm and 30-60 cm respectively. Soil texture was silty clay loam at both soil depths.

To measure green fruit yield, five plants were randomly chosen at the beginning of the first harvest in each experimental unit, and the next measurements were carried out in the same plants the following harvests. Green fruit yield per ha was estimated considering 31,250 plants in this area. Fruit length and fruit width was measured randomly in 25 plants in each experimental unit and the average was analyzed. In 2013, plots were harvested June 20, July 5, July 20, August 5, August 20, and September 5. In 2014, plots were harvested July 4, July 25, August 15, August 28, and September 10.

RESULTS

Green fruit yields

There was no difference among split plots fertilization treatments on total fruit

yield in 2013 ($p > 0.05$), and the highest fruit yield was found in the 40 t ha⁻¹ manured treatment in 2014 ($p < 0.05$) (table 1).

Total fruit yields of the whole plots treatments showed difference in 2013 and 2014 ($p < 0.05$), the drip irrigation treatment was significantly higher than the furrow irrigation treatment. Respect to the fruit yield per harvest, the split plot fertilized treatments showed no differences ($p > 0.05$) in 2013 (table 2, page 110).

In the first harvest of 2014, the 40 t ha⁻¹ manured treatment was higher than the 20 t ha⁻¹ manured treatment and similar to the other treatments ($p < 0.05$). In the second harvest, the 40 t ha⁻¹ manured treatment was higher than the control and the 20 t ha⁻¹ manured treatment and similar to the chemical treatment ($p < 0.05$). In the third and fourth harvests there were no differences among fertilized treatments ($p > 0.05$).

Table 1. Green fruit yield, fruit length, and fruit width of *Capsicum annum* L. of the periods evaluated at Facultad de Agricultura y Zootecnia, UJED, Gómez Palacio, Dgo. Mexico.

Tabla 1. Producción de fruto verde, longitud y ancho del fruto de *Capsicum annum* L. en los períodos evaluados en la Facultad de Agricultura y Zootecnia, UJED, Gómez Palacio, Dgo. Mexico.

	---green fruit yield---		----fruit length----		---fruit width---	
	(t ha ⁻¹)		(cm)		(cm)	
	2013	2014	2013	2014	2013	2014
-----sub-plot fertilized treatments-----						
M40 ¹	50.98 a ³	44.95 a	6.30 a	4.64 b	2.17 a	2.66 a
M20	50.53 a	34.60 b	6.06 b	4.82 a	2.13 ab	2.53 a
ChT	52.03 a	37.91 ab	6.26 a	5.08 a	2.12 ab	2.65 a
CT	47.02 a	35.05 b	6.21 a	5.10 a	2.07 b	2.53 a
-----Whole plots, irrigation treatments-----						
DI ²	56.61 a	41.41 a	6.42 a	5.04 a	2.18 a	2.63 a
FI	43.67 b	34.84 b	5.99 b	4.76 a	2.06 a	2.55 a

¹M40 = 40 t ha⁻¹ of solarized manure, M20 = 20 t ha⁻¹ of solarized manure, ChT = 160-80-00 of NPK kg, CT = without any fertilized treatment. ²DI = drip irrigation. FI = furrow irrigation. ³Means with different letter in the same column show significant differences ($p < 0.05$).

¹M40 = 40 t ha⁻¹ de estiércol solarizado, M20=20 t ha⁻¹ de estiércol solarizado, ChT = tratamiento químico 160-80-00 de NPK kg, CT = sin tratamiento de fertilización. ²DI = riego por goteo, FI = riego por surcos.

³Medias con letra diferente en la misma columna muestran diferencia significativa ($p < 0,05$).

Table 2. Green fruit yield per harvests of *Capsicum annuum* L. in the subplot and the whole plot treatments.

Tabla 2. Producción de fruto verde por cosecha de *Capsicum annuum* L. en los tratamientos de las subparcelas y parcelas grandes.

	Y̅1 ¹		Y̅2		Y̅3		Y̅4		Y̅5		Y̅6	
Subplots ²	----- Green fruit yield (t ha ⁻¹) 2013 -----											
M40	5.27	a ³	9.87	a	7.68	a	9.71	a	9.73	a	9.06	a
M20	5.26	a	11.34	a	6.24	a	9.31	a	8.40	a	11.22	a
ChT	4.71	a	10.08	a	8.30	a	9.52	a	9.60	a	10.34	a
CT	4.09	a	10.92	a	6.24	a	9.75	a	8.43	a	8.94	a
Subplots	----- Green fruit yield (t ha ⁻¹) 2014 -----											
M40	9.17	a	9.42	a	6.48	a	9.41	a	9.60	a		
M20	6.24	b	7.12	b	5.58	a	7.37	a	7.17	b		
ChT	7.21	ab	7.69	ab	6.99	a	7.70	a	8.04	ab		
CT	7.27	ab	7.13	b	6.75	a	7.02	a	7.40	b		
Whole plots ⁴	----- Green fruit yield (t ha ⁻¹) 2013 -----											
DI	6.37	a	12.13	a	9.04	a	10.03	a	10.54	a	9.91	a
FI	3.29	b	8.96	a	5.97	b	9.14	a	7.82	a	9.96	a
	----- Green fruit yield (t ha ⁻¹) 2014 -----											
DI	8.12	a	8.37	a	6.74	a	8.60	a	8.81	a		
FI	7.04	a	7.29	a	6.18	a	7.10	b	7.35	b		

¹ Mean of the harvest number one. ² M40 = 40 t ha⁻¹ of solarized manure, M20 = 20 t ha⁻¹ of solarized manure, ChT = Chemical treatment 160-80-00 of NPK kg, CT = without any fertilized treatment. ³ Means with different letter in the same year and column show significant differences (p<0.05). ⁴ DI = drip irrigation, FI = furrow irrigation.

¹ Media de la cosecha número uno. ² M40=40 t ha⁻¹ de estiércol solarizado, M20 = 20 t ha⁻¹ de estiércol solarizado, ChT = tratamiento químico 160-80-00 de NPK kg, CT = sin tratamiento de fertilización.

³ Medias con letra diferente en el mismo año y columna muestran diferencia significativa (p<0,05).

⁴ DI = riego por goteo, FI = riego por surcos.

In the whole plot treatments, fruit yield was greater in drip irrigation treatment than furrow irrigation only in the first and third harvests in 2013, and in the fourth and fifth harvests in 2014 (p<0.05).

In the other harvests no differences were found (p>0.05) (table 2).

Fruit length and fruit width

In the whole-plot treatments, the greatest fruit length was found in drip irrigation treatment in 2013 (p<0.05) and there was no difference between treatments in 2014 (p>0.05) (table 1, page 109). The 20 t ha⁻¹ manured treatment showed the lowest fruit length in the split

plot treatments (p<0.05) in 2013. In 2014, the 40 t ha⁻¹ manured treatment showed lower fruit length than the other fertilized treatments (p<0.05) (table 1, page 109).

In fruit diameter, the 40 t ha⁻¹ manured treatment showed no differences with the other treatments in 2013, and having greater fruit diameter than the control treatment (p<0.05) (table 1, page 109). The chemical, the 20 t ha⁻¹ manured and control treatments showed similar diameter fruit (p>0.05). There was no difference in this variable among split plot fertilized treatments in 2014 (p>0.05). In the whole plots there was no difference between treatments in 2013 and 2014 (p>0.05).

Soil organic matter

In 2103, in the split plot fertilized treatments, the 20 and 40 t ha⁻¹ manured treatments had similar amount of soil organic matter found at a soil depth of 0-30 cm ($p>0.05$). The 20 t ha⁻¹ manured treatment showed greater amount of soil organic matter than the other treatments in this soil depth ($p<0.05$) (figure 1, page 112). At the same soil depth, the 40 t ha⁻¹ manured treatment was similar to the control treatment and greater than the chemical treatment in this variable ($p<0.05$). The control and chemical treatments had similar amount of soil organic matter at that same soil depth ($p>0.05$). In 2014, no differences were found among split plot fertilized treatments at the soil depth before mentioned ($p>0.05$).

In the whole plots there was no significant difference of organic matter in 2013 and 2014 at this soil depth of 0-30 cm ($p>0.05$). There were no significant differences of soil organic matter at a depth of 30-60 cm for neither the split plot fertilizer treatments nor the whole plot irrigation treatments ($p>0.05$) (figure 1, page 112).

Nitrates, pH and electrical conductivity (EC)

The 40 t ha⁻¹ manured treatment had the greater amount of nitrates than the other treatments ($p<0.05$) at the 0-30 cm soil depth level in 2013. In 2014 there were no differences among split plot fertilization treatments ($p>0.05$). In the whole plots, drip and furrow irrigation, there was no difference at this soil depth in both years ($p>0.05$) (figure 1, page 112).

In the split plot fertilized treatments, the chemical treatment was similar to the manured treatments and greater than the control treatment ($p<0.05$) in nitrates concentration at soil depth of 30-60 cm, and control treatment showed

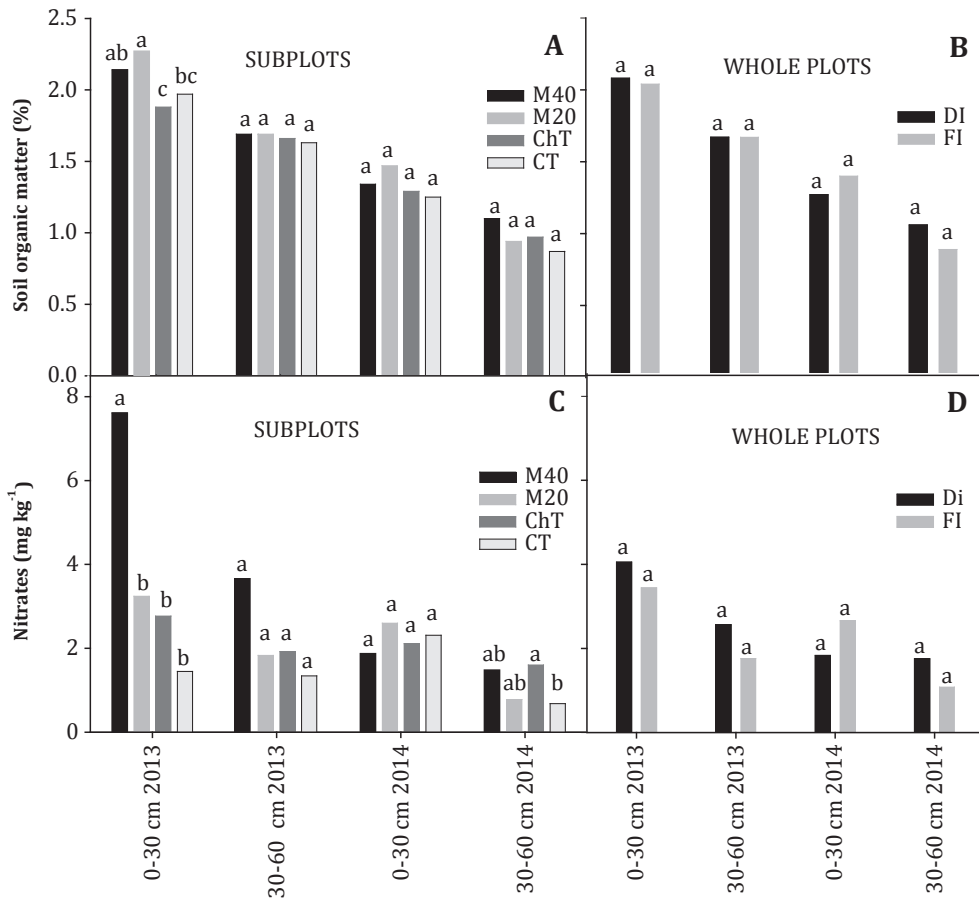
no differences with the manured treatments ($p>0.05$). In the whole plot treatments there was no difference in nitrates at this soil depth (figure 1, page 112). Significant difference was not found neither for split plot fertilized or whole plot irrigation treatments in pH and EC at 0-30 cm soil depth level ($p>0.05$) (figure 2, page 113).

DISCUSSION

Green fruit yield

Assouline *et al.* (2006), in order to investigate irrigation frequencies (up to 10 per day), found that the highest values of yield (5 kg m⁻²), efficiency of water use (10.7 kg m⁻³) was achieved with a daily irrigation drip, because the frequencies generated higher irrigation crop damage by excessive accumulation of salts in the rhizosphere. In the same species, Sezen *et al.* (2006) found that the highest yield of fresh fruit (33.1 t ha⁻¹) was obtained when irrigation was applied to achieve a consumption of 18-22 mm of evaporation of a standard tank type "A", with a coefficient of development crop equals one.

These green fruit yields are similar to those found by Vázquez-Vázquez *et al.* (2011) and higher than those reported by Ashrafuzzaman *et al.* (2011). These results were in a certain way because of the jalapeño hybrid 'Compadre' is one of the chili crops that are cultivated in the Center-North region of Mexico which have higher fruit yields than those hybrids that are cultivated in the Mexico South-Gulf region (2). When the amount of fertilizer that the plant needs is applied to the soil instead of recommended applications in a certain region, yield results are better without N losses (10).

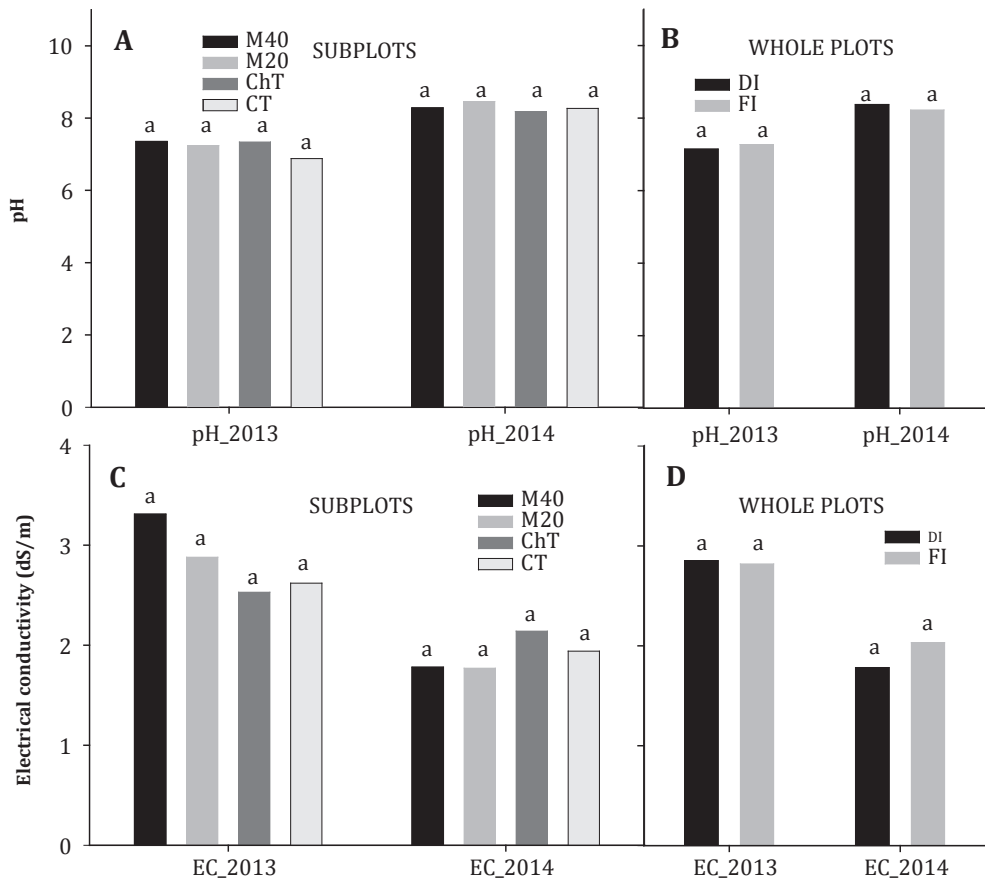


M40 = 40 t ha⁻¹ of solarized manure. M20 = 20 t ha⁻¹ of solarized manure. ChT = chemical treatment 160-80-00 kg of NPK. CT = without any fertilized treatment. DI = drip irrigation. FI = furrow irrigation. Means with different letter in the same column (date) show significant differences (p<0.05).

M40 = 40 t ha⁻¹ de estiércol solarizado. M20 = 20 t ha⁻¹ de estiércol solarizado. ChT = tratamiento químico 160-80-00 kg de NPK. CT = sin fertilizante. DI = riego por goteo. FI = riego por surcos. Medias con letra diferente en la misma columna (fecha) muestran diferencia significativa (p<0,05).

Figure 1. Soil organic matter (A and B) and nitrates (C and D) found after jalapeño pepper was harvested.

Figura 1. Materia orgánica del suelo (A y B) y nitratos (C y D) encontrados después de que el chile jalapeño fue cosechado.



M40 = 40 t ha⁻¹ of solarized manure. M20 = 20 t ha⁻¹ of solarized manure. ChT = chemical treatment 160-80-00 of NPK kg. CT = without any fertilized treatment. DI = drip irrigation. FI = furrow irrigation. Means with similar letter in the same column and year show no significant differences (p>0.05).

M40 = 40 t ha⁻¹ de estiércol solarizado. M20 = 20 t ha⁻¹ de estiércol solarizado. ChT = tratamiento químico 160-80-00 de NPK kg. CT = sin fertilizante. DI = riego por goteo. FI = riego por surcos. Medias con letras iguales en la misma columna y año no muestran diferencias significativas (p>0,05).

Figure 2. pH (A and B) and electrical conductivity (EC, C and D) at the depth soil of 0-30 cm after the jalapeno pepper was harvested in 2013 and 2014.

Figura 2. pH (A y B) y conductividad eléctrica (EC, C y D) del suelo a 0-30 cm de profundidad después de que el chile jalapeño fue cosechado en 2013 y 2014.

In this way, the highest fruit yield found in 2014 is according to the results found by Vázquez-Vázquez *et al.* (2011) who found the best yield with the application of 40 t ha⁻¹ of manure. It seems that in the first year (2013) manure and chemical fertilizers had no effects on green fruit yields, but in the second year (2014) of the manure and chemical fertilizer application an accumulative effect was showed in the fruit yield.

Fruit length and fruit width

Sermenli and Mavi (2010) reported similar fruit length results. Ashrafuzzaman *et al.* (2011) and Gunawardena and De Silva (2014) showed lower length fruit than this research and Jayasree and George (2006) found greater length fruit of jalapeño pepper. Also, Sermenli and Mavi (2010) found similar results in fruit width. Ashrafuzzaman *et al.* (2011) and Gunawardena and De Silva (2014) showed lower fruit width in research they carried out.

Organic matter

Soil organic matter is an important factor for providing nutrients supply (19), especially nitrogen (17), but it is known that the nutrients of the organic matter are not available for plants at the beginning (30). In the first year of manure application, the results in fruit yield were similar to the control treatment and there was more residual soil organic matter in the manured treatments.

In the second year of manure application, equal percentages of residual organic matter in the soil were found among the treatments and the 40 t ha⁻¹ manured treatment had the greatest fruit yield what it indicates that organic matter was more useable by plants trough net N mineralization process in the soil (12), and repeated applications of farmyard manure increase the N availability for plants (8).

Nitrates

The nitrates had a similar tendency that soil organic matter. There were more soil nitrates in the first year in the manured treatments after the fruit yield was done, but this fruit yield did not show a difference with the control treatment, what it could indicate that nitrates were no available to be used by plants at that time (30), but in the second year the soil nitrates were lower at the end of the last harvest than the first year in the manured treatments, what it could indicate that nitrates were used by plants to get more fruit production.

In the second year, the results show that fruit yield was greater in the 40 t ha⁻¹ manured treatment and the depletion of nitrates found in the soil indicates that N was used by plants instead of being lost by denitrification.

The reason why people adds manure is due to its low C:N ratio where microorganisms do not have any trouble to mineralize N and plant available-N increases in soil (7, 24), although soil temperature, water content, and drying and rewetting events must be considered in this process (5), and C:N ratios of crop residues should be noted to avoid N losses after harvest (10, 25).

pH and Electrical Conductivity (EC)

Because of the higher fruit yield in the 40 t ha⁻¹ manured treatment, a higher soil pH and a lower soil electrical conductivity in 2014, it does not seem that these conditions allowed a soil denitrification (29).

The irrigation every four days applying 60% of water evaporated in this study had no adverse effects in fruit yield, because both high electrical conductivity and water-filled pored space affect positively soil denitrification (1, 6).

CONCLUSIONS

There were a better jalapeño fruit yields by using drip irrigation because of frequency of water application according to the water evaporation, therefore the first hypothesis of this research was accepted.

The 40 t ha⁻¹ manured treatment had a greater fruit yield until the second year of

the continuous application of this amount of manure, the other hypothesis accepted. This result could be consequence that nitrates were taken up by the plants in lower amount in the first year what it was indicated by residual amounts of nitrates in the soil.

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The effect of citric acid on the phenolic compounds, flavonoids and antioxidant capacity of wheat sprouts

Efecto del ácido cítrico sobre los compuestos fenólicos, los flavonoides y la capacidad antioxidante en brotes de trigo

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ABSTRACT

Wheat sprouts are a source of carbohydrates, fiber, vitamins, essential nutrients and bioactive compounds that have been linked to the prevention and treatment of illnesses. The presence of these compounds in wheat sprouts may be increased by means of the application of organic acids during the growth stage. The objective of this investigation was to evaluate the effect of a sprayed application of citric acid (0, 10⁻², 10⁻³, 10⁻⁴ and 10⁻⁵ M) on the total antioxidant capacity and total content of phenolic compounds and flavonoids in wheat sprouts. A completely randomized design was used, consisting of six repetitions. The results showed that phenolic compounds, flavonoids and antioxidant capacity increased significantly with the application of a 10⁻² M foliar spray as compared to the control. As such, the spraying of low concentrations of secondary metabolism-inducing molecules during the sprout development process is a useful and effective method to stimulate the biosynthesis of bioactive phytochemicals and improve the nutraceutical quality of wheat sprouts.

Keywords

Nutraceutical quality • nutrition • food • germinated

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RESUMEN

Los brotes o germinados son una fuente de carbohidratos, fibra, vitaminas, nutrimentos esenciales y compuestos bioactivos, los cuales se han relacionado con la prevención y tratamiento de enfermedades. La presencia de estos compuestos, en brotes de trigo, pueden incrementarse mediante la aplicación de ácidos orgánicos durante la etapa de crecimiento. El objetivo de la presente investigación fue evaluar el efecto de la aplicación por aspersión de ácido cítrico (0 , 10^{-2} , 10^{-3} , 10^{-4} y 10^{-5} M) en la capacidad antioxidante total, contenido de compuestos fenólicos totales y flavonoides totales en brotes de trigo. Se utilizó un diseño completamente al azar con seis repeticiones. Los resultados mostraron que los compuestos fenólicos, flavonoides y la capacidad antioxidante se incrementaron significativamente con la aspersión foliar 10^{-2} M en relación al testigo. Por lo tanto, la aspersión de bajas concentraciones de moléculas inductoras del metabolismo secundario durante la etapa de desarrollo de brotes es un método útil y efectivo para estimular la biosíntesis de fitoquímicos bioactivos y mejorar calidad nutracéutica de los brotes de trigo.

Palabras clave

calidad nutracéutica • nutrición • alimento • germinados

INTRODUCTION

Wheat sprouts are a fresh food product that provides carbohydrates, fiber, vitamins, nutrients and a high content of phytochemical compounds with a bioactive effect and "nutraceutical" qualities, including antibacterial, anti-diabetic, anti-inflammatory, anti-cancer and antioxidant effects (6, 17). Wheat sprouts are widely consumed in countries such as Spain, Japan and the United Kingdom due to their quality and composition; however, they are not commonly consumed as part of the gastronomic culture in developing countries (21). On the other hand, these countries present high rates of malnutrition (11) and chronic degenerative illnesses such as obesity, diabetes and hypertension (18). Due to the above, societies increasingly demand foods that not only satisfy hunger, but that also contain bioactive compounds with antioxidant effects (5, 22). The antioxidant effect of foodstuffs is

related to their capacity to slow or prevent the oxidation of biological molecules, preventing their oxidation and reducing aging and the emergence of illnesses (7). In this sense, it is prudent to develop technologies that increase the antioxidant content and overall production of foods, which can be of use to both the food and pharmaceutical industries (8, 9). Several studies have demonstrated that the nutraceutical properties of sprouts of various species can be increased with the application of compounds that function as elicitors, promoting the synthesis of derivative compounds of phenylpropanoids and activating signal cascades that increase antioxidant activity (28). The application of hydrogen peroxide, yeast hull extracts, various abiotic stresses and minerals such as selenium and sulfur are all treatments that have been studied in relation to improving the content of health-promoting compounds (27).

The application of organic acids has also been demonstrated to increase the presence of the compounds in question (30). As the subject of this study, citric acid has been shown to increase the antioxidant content of foods, being that it stimulates greater synthesis of nutraceutical compounds that function as antioxidants, including phenolic compounds, flavonoids and tannins (15, 23). As such, the application of citric acid during the production of wheat sprouts could be a useful tool to increase the synthesis of bioactive compounds. Wheat seeds contain high levels of nutrients and antioxidants (13), presenting moderate levels of phenolic compounds that complement those found in fruits. On the other hand, no studies are currently available that have explored the effect of citric acid applications on the nutraceutical quality of wheat sprouts. As such, the objective of this investigation was to evaluate the effect of a sprayed application of citric acid in varying concentrations on total antioxidant capacity and the total content of phenolic compounds and flavonoids in wheat sprouts.

MATERIALS AND METHODS

The study was carried out in the Biotechnology Laboratory in the Gómez Palacio Polytechnical University, located in in Gómez Palacio, Durango, Mexico. Wheat seeds (*Triticum aestivum L*) were used, with a germination percentage of 95%. Temperature conditions were 15-18°C. Distilled water was used through the study. Four stages can be identified in the process of obtaining sprouts: washing and disinfection, pregermination, germination and sowing, and growth. In the first stage, seeds were submitted to washing

through immersion in water containing sodium hypochlorite (NaClO , 1 mL L^{-1}) for 15 minutes for the purpose of disinfection. Following this, the seeds were rinsed twice in order to remove excess NaClO and drained prior to pregermination.

The pregermination phase consisted of immersing the seed in water for 6 hours (6). Next, the seeds were drained and rinsed prior to germination. The germination stage consisted of placing the pregerminated seeds in $15 \times 10 \times 5 \text{ cm}$ polystyrene foam containers, perforated in the base to allow percolation and aeration. The containers were covered with a number one cloth and placed in darkness. This stage lasted 6 hours. After this time began the growth stage, for which the cloth was removed and the containers were placed on shelves with natural lighting. In this stage the treatments were applied and which lasted 6 days.

A completely randomized experimental design with six repetitions per treatment, consisting of the application of citric acid ($\text{C}_6\text{H}_8\text{O}_7$) in concentrations of 0 , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} M (1) was used. During the germination and sowing stage irrigation was applied every 3 hours, exclusively with water, through the spraying of 3 ml per application. The application of the treatments took place at the growth stage with the same irrigation dosage. The variables evaluated were total antioxidant capacity (TAC), total phenolic content (TPC), and total flavonoid content (TFC).

Extracts were prepared in order to quantify the variables evaluated in the study, mixing 2 g of fresh sample in 10 ml of ethanol at 80 % in screw-top plastic tubes which were placed in a rotating agitator (ATR Inc., US) for 24 hours at 20 rpm and 5°C. The tubes were then centrifuged at 3000 rpm for 5 minutes, and the overdrain extracted for analysis.

The TAC was determined with the *in vitro* DPPH⁺ method (2, 3). To carry out the method, a solution of DPPH⁺ (Aldrich, St. Louis, Missouri, USA) in ethanol was prepared, adjusting the solution absorbance to 1.100 ± 0.010 at a wavelength of 515 nm. A standard curve was obtained with Trolox (Aldrich, St. Louis, Missouri, EU), and the results were reported as equivalent antioxidant capacity in μM equivalent in Trolox per 100 g base fresh weight (M equiv Trolox \cdot 100 gm⁻¹FW).

The TPC was determined with a modified Folin-Ciocalteu method (26). To calculate TPC, a calibration curve was created using gallic acid as a standard, and the results were registered as mg of gallic acid equivalent per 100 g base fresh weight (mg GAE/100 g FW). To quantify the concentration, a calibration curve was created using a quercetin standard. The results were expressed as mg quercetin equivalent per 100 g base fresh weight (mg g⁻¹ FW).

For the statistical analysis, the SAS software version 9.0 (25) was used. A Tukey test at 0.05 probability was applied for the comparison of averages. Furthermore, the coefficient of determination analysis ($p \leq 0.05$) was made in order to detect association between the evaluated variables.

RESULTS AND DISCUSSION

Different concentrations of citric acid led significant differences ($p \leq 0.05$) in TAC in the wheat sprouts. A concentration of 10^{-2} M provoked the greatest antioxidant capacity, whereas the lowest value was obtained with the 10^{-4} to 10^{-5} M solution (table 1). The antioxidant capacity of a foodstuff depends on the nature and concentration of the different compounds and the natural antioxidants present within (4). In the case of this study, the antioxidant capacity can be attributed to the action of the citric acid in stimulating the accumulation of antioxidant compounds (16).

On the other hand, Swieca and Baraniak (2014) highlight that greater or lesser antioxidant activity is not always accompanied by the concentration of phenolic compounds. These authors stress that, when capacity for free radical capture is being assessed, TPC is not always as important as the position of the hydroxyl group. Another factor that may increase antioxidant activity in wheat sprouts are the additive and synergetic affects between other phytochemicals and phenolic compounds altered by the elicitation effect of citric acid.

Table 1. Average values obtained in wheat sprouts under different molar concentrations of citric acid.

Tabla 1. Valores promedio obtenidos en brotes de trigo bajo diferentes concentraciones molares de ácido cítrico.

Citric Acid M	TAC	TPC	TFC
	Meq TOLOX/100 gr PF	mg AGE/100 gr PF	mg QE/100 gr PF
10^{-2}	115.04 a	189.97 a	150.03 a
10^{-3}	105.56 b	154.27 b	138.62 b
10^{-4}	99.50 d	142.70 c	129.84 c
10^{-5}	99.73 d	136.22 d	112.91 d
0	100.93 c	136.33 d	102.17 e

In order to determine the relationship between antioxidant capacity and phenolic compound content, in this study the coefficient of determination analysis was performed (figure 1A, page 124), in which it was shown that there was a strong positive correlation between both variables ($r^2=0.96$), suggesting that these phenolic compounds are antioxidant in nature. This correlates with other studies in which it was found that the potential health benefits of phenolic compounds can be primarily attributed to their antioxidant activity through the donation of a hydrogen atom from the aromatic hydroxyl group to the free radicals (14).

Regarding the TPC, a significant increase was found that can be attributed to the application of citric acid ($p\leq 0.05$). The 10^{-2} M treatment surpassed the control treatment by 72 % (table 1, page 122).

The lowest concentration of TPC was obtained with the lowest citric acid dosage (10^{-5}) and the control. While there continues to be a deficit of studies into the effect of citric acid application, results can be found that indicate the possibility of obtaining an increase in phenolic compound content due to the effect of treatments that cause the degradation of polyphenols such as tannins to simple phenols (16). It is also known that some phenolic compounds accumulate in cellular vacuoles (29), and that hydrolyzation by citric acid can liberate these otherwise unavailable phenols.

The increase found as a result of the citric acid application may be attributable to the liberation of phenolic acids following the decomposition of cellular components and walls. This suggests a disassociation of polyphenolic forms due to citric acid hydrolyzation, possibly followed by aglycosylation, polymerization or oxidation of the phenolic components (30), which was responsible for the increase in total

phenolic compound content found in this study. Similarly, the coefficient of determination was made between the TPC and that of the flavonoids (figure 2, page 125), as these were the principal phenolic compounds identified and reported in the sprouts (24). The results showed a positive correlation between the TPC and the TFC ($r^2=0.92$), demonstrating that the flavonoids belong to the polyphenolic group (14).

TFC found in the wheat sprouts was significantly affected by the different concentrations of citric acid ($p\leq 0.05$). The values obtained fluctuated between 102 and 150 mg of quercetin (QE) / 100 g fresh weight (table 1, page 122). The greatest flavonoid content was found in the wheat sprouts that were sprayed with a 10^{-2} concentration of citric acid, compared to the control in which a value 68 % smaller was obtained.

Previous studies indicate that application of citric acid increases the synthesis of secondary metabolites such as flavonoids (19), which correlates with the results of the present study, in which the citric acid application considerably increased the presence of these metabolites. It is possible that a synergistic reaction occurred with the application of the acid, which activated the transduction pathways that conduct the secondary signals produced by plants (20).

Several investigations have shown that flavonoids are implicated in the prevention of chronic non-transmissible diseases due to their antioxidant activity (10), which agrees with the correlation obtained in the current study (figure 1B, page 124) between antioxidant capacity and flavonoid content ($r^2=0.97$). This suggests that flavonoids may be implicated in the prevention of chronic non-transmissible diseases as a result of their antioxidant activity (12).

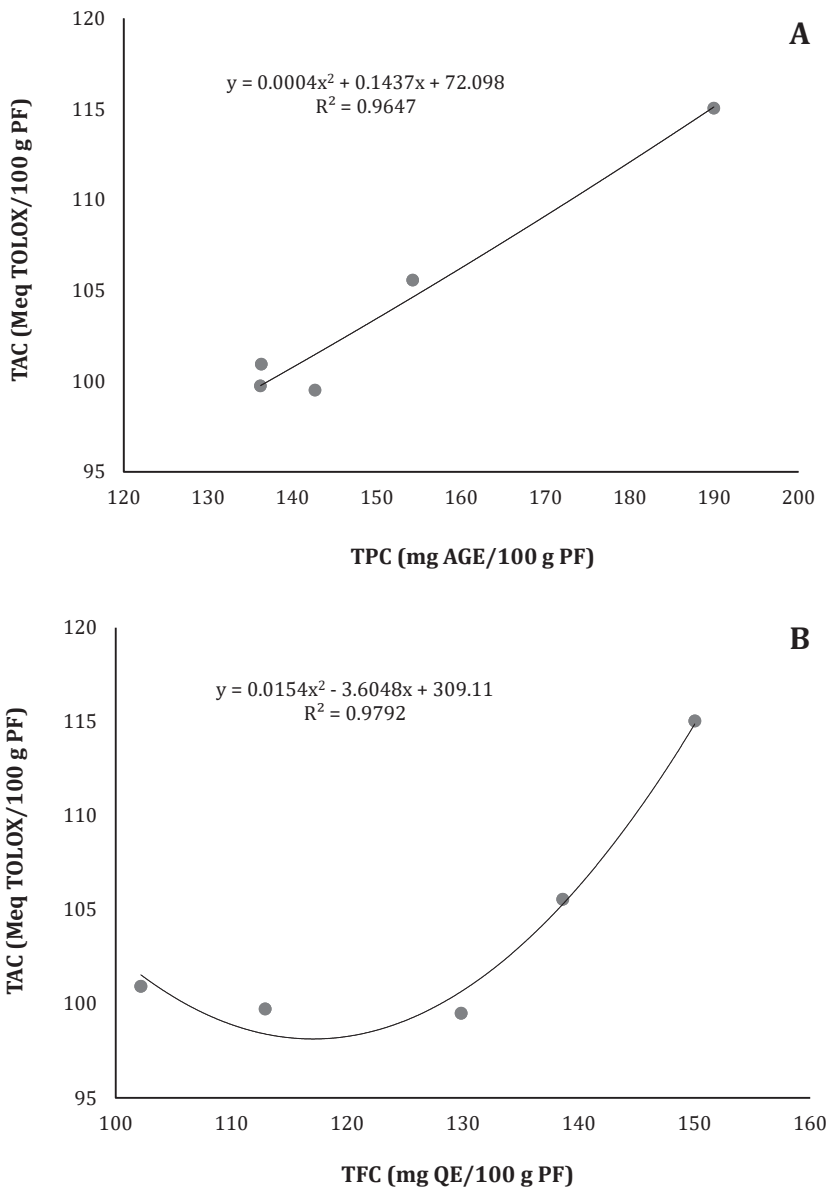


Figure 1. A) Pearson correlation (r^2) between total antioxidant capacity (TAC) with total phenolic content (TPC) and B) total antioxidant capacity (TAC) with total flavonoid content (TFC) in wheat sprouts under different concentrations of citric acid.

Figura 1. A) Coeficientes de determinación (r^2) entre la capacidad antioxidante total (CAT) con los compuestos fenólicos totales (CFT), y B) capacidad antioxidante total (CAT) con el contenido de flavonoides totales (FT) en brotes de trigo bajo diferentes concentraciones molares de ácido cítrico.

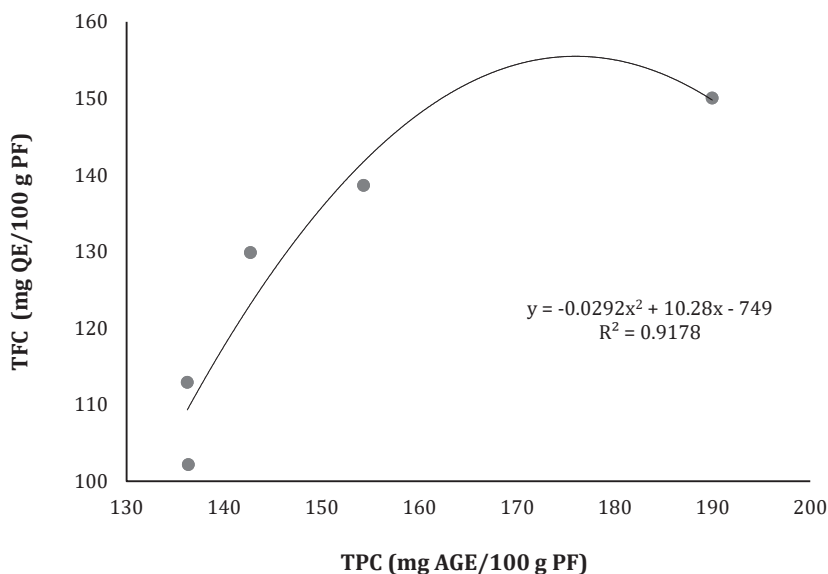


Figure 2. Pearson correlation (r^2) between total phenolic content (TPC) and total flavonoids (TFC) in wheat sprouts under different concentrations of citric acid.

Figura 2. Coeficientes de determinación (r^2) entre el contenido de compuestos fenólicos totales (CFT) y flavonoides totales (FT) en brotes de trigo bajo diferentes concentraciones molares de ácido cítrico.

The results suggest a positive increase in each variable evaluated by the application of citric acid. However, it is not conclusive that the content of phenolic compounds, flavonoids and the subsequent antioxidant capacity in the evaluated germinates can always be linear with the increase in the application of citric acid.

Therefore, it could be interesting to investigate the range of application of this acid to determine if there is a quadratic response and to relate the molar concentration of citric acid and the production of phenolic compounds and total flavonoids. Likewise, it remains to be resolved if the increase in the concentration of citric acid modifies other quality variables and the development of the sprouts.

CONCLUSIONS

The total phenolic and flavonoid content of the wheat sprouts increased significantly as a result of the 10^{-2} M citric acid solution treatment, by 205 % and 184%, respectively, while the TAC increased by 123 %.

As such, citric acid favors the biosynthesis of phytochemical compounds in wheat sprouts when sprayed during the growth stage.

However, it could be interesting to increase the range of application of this acid to determine if there is a quadratic response and to relate the molar concentration of citric acid and the production of phenolic compounds and total flavonoids.

These results indicate that the application of low concentrations of secondary metabolism-inducing molecules is a useful and effective means of stimulating the biosynthesis of bioactive phytochemicals

and improving the antioxidant capacity of wheat sprouts, opening up the possibilities of using this ingredient for consumption in functional foodstuffs.

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Effect of *Prosopis* spp. as a nurse plant on nutrient content and productivity of *Opuntia ellisiana* Griffiths

Efecto de *Prosopis* spp. como planta nodriza sobre el contenido de nutrientes y la productividad de *Opuntia ellisiana* Griffiths

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ABSTRACT

In arid conditions, the low quality of the forage and water shortage could be attenuated by the introduction of *Opuntia* species. Several plant species grows well under *Prosopis* canopy, responding to a higher nutrient content in the soil. It was hypothesized that productivity and nutrient content, mainly crude protein (CP), of *Opuntia ellisiana* planted under *Prosopis* would be higher than of those planted outside the canopy, and that these parameters would be influenced by the cactus position: north or south from the centre of the tree. Sampling was done in 18 plants under the canopy of *Prosopis* and 23 outside of it. After one year of the plantation, the totality of cladodes was harvested. The productivity per plant was 3.1 and 5.7 cladodes and 34.9 and 48.1 g of dry matter (DM), outside and under the canopy, respectively. The bromatological values (%) were moisture: 89.1 and 91.9, organic matter (OM): 76.7 and 79.8, CP: 4.4 and 8.1, acid detergent fiber (ADF): 13.7 and 18.2, neutral detergent fiber (NDF): 29.0 and 31.5, K: 3.1 and 3.6, P: 0.07 and 0.11, Ca: 5.5 and 4.4, Na: 0.04 and 0.02, outside and under the canopy, respectively. Crude protein, moisture, OM, ADF, NDF, K, P and productivity per plant were higher under the canopy, whereas Ca and Na values were higher outside the canopy ($p < 0.05$). Magnesium (1.9 and 1.9%) was not affected by the position outside or under the canopy. The north or south orientation did not influence any of the analysed parameters.

Keywords

bromatological composition • crude protein • food reserve • agrosystem enrichment • arid lands

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RESUMEN

En condiciones de aridez, la baja calidad del forraje y la escasez de agua podrían ser atenuadas con la introducción de especies de *Opuntia*. Varias especies tienen buen crecimiento bajo la canopia de *Prosopis* spp., respondiendo a un contenido más alto de nutrientes en el suelo. Se hipotetizó que la productividad y el contenido de nutrientes, principalmente proteína cruda (PC), de *Opuntia ellisiana* establecidas bajo la canopia de *Prosopis* serían más altos que los implantados fuera de la misma y que estos parámetros serían influenciados por la posición del cactus: norte o sur respecto del centro del árbol. Se muestrearon 18 plantas de cactus bajo la canopia y otras fuera de la misma. Después de un año de crecimiento, se cosechó la totalidad de los cladodios. La productividad por planta fue 3,1 y 5,7 cladodios y 34,9 y 48,1 g de materia seca (MS), fuera y bajo la canopia, respectivamente. Los valores bromatológicos (%) fueron humedad: 89,1 y 91,9, materia orgánica (MO): 76,7 y 79,8, PC: 4,4 y 8,1, fibra detergente ácida (FDA): 13,7 y 18,2, fibra detergente neutra (FDN): 29,0 y 31,5, K: 3,1 y 3,6, P: 0,07 y 0,11, Ca: 5,5 y 4,4, Na: 0,04 y 0,02, bajo y fuera de la canopia, respectivamente. Proteína cruda, humedad, MO, FDA, FDN, K, P y productividad por planta fueron más altas bajo la canopia, mientras que los valores de Ca y Na fueron más altos fuera de la canopia ($p < 0.05$). Magnesio (1,9 y 1,9%) no fue afectado por la posición bajo y fuera de la canopia, respectivamente. La orientación norte o sur no influenció los parámetros analizados.

Palabras clave

composición bromatológica • proteína cruda • reserva de alimento • enriquecimiento del agroecosistema • tierras áridas

INTRODUCTION

There are numerous reasons behind the diffusion of cacti as forage or fodder around the world, such as the simple cultivation practices required to grow the crop, rapid establishment in a new area, easy multiplication practices (18). Also they have the ability to withstand prolonged drought, high temperatures as well as soils affected by wind and water erosion (16). This aptitude has made the cactus plantations suitable as food supply and they have become strategic to mitigate the effect of drought over animal production systems in various arid and semi-arid areas of the world. Cacti have greater water-use efficiency due to Crassulacean Acid Metabolism (CAM) photosynthetic pathway (24) and this makes them

especially suited for forage productions in arid lands.

Opuntia species are not a balanced feed: rich in energy, calcium and beta carotene, but poor in fiber and nitrogen (4). As evidenced by various studies, some efforts have been made with the purpose of increasing the nitrogen content in cactus for using as forage (8, 10, 17).

Opuntia spp. can be cultivated in a wide range of environments. The major limitation to cultivation of cactus in many areas of the world are severe cold winter temperatures such as happens in the region of Mendoza, Argentina (16), northern Mexico (5), the Mediterranean Basin (22), the arid highland steppes

of western Asia (23) and the south-western United States (25). *O. ellisiana* has a lower growth and productivity compared to other *Opuntia* species such as *O. ficus-indica* (L.) Mill. but it stands out for its resistance to sub-zero temperatures proper of the arid lands.

Prosopis spp. has the capacity of tolerating drought and unfavourable edaphic conditions, such as salinity, as well as being well adapted to the herbivory. All these are the main reasons of its dominant position in the silvopastoral systems of the arid and semi-arid areas of America. On the other hand, they generate spatial heterogeneity that affects the distribution of the shrub and herbaceous layers due to the modification of the microclimatic conditions under its canopy (29).

The improvement in the forage value of cactus under the *Prosopis* crown indicates the better condition of the site as a result of the higher nutrient content of the soil (26). In arid ecosystems, dominant woody plants are likely to cause changes in microclimate and soil properties by mitigating harsh environmental conditions such as high temperature and solar radiation (6, 19). Also, shrub species play an important role in arid land vegetation, since in several cases, they act as "nurse plants" improving microclimatic conditions, increasing water and nutrient availability, and offering protection against herbivory (13).

The objective of this work was to determine the effect of associating tree-cactus over the bromatological parameters and DM productivity per plant of *Opuntia ellisiana* Griffiths, implanted under and outside the *Prosopis* spp. canopy, considering the north and south location respect to the tree. It was hypothesized: a) productivity and nutrient content, mainly crude protein (CP), of cactus under the *Prosopis* will be greater

than those planted outside the canopy and b) these parameters will be influenced by the location of cacti plantation, north or south, with respect to the *Prosopis* trunk.

MATERIALS AND METHODS

The experience was carried out in the campus of the CCT-CONICET-Mendoza (32°53'45" S; 68°52'28" W, 840 m a. s. l.) Mendoza, Argentina. The maximum and minimum absolute temperatures (2014-2015 period) were 38.0 and 0.9°C in summer and winter, respectively. Precipitation is characterized by high spatial and temporal variability, it occurs in summer mostly as intense precipitations and the mean for the 2014 and 2015 period was 324.2 mm year⁻¹, during an exceptionally wet period given that the mean of the past 10 years was 226.7 mm year⁻¹. Soils in the study area are of alluvial origin with heterometric clasts in a sandy matrix, normally non-structured, deep, well drained, belonging to the order of Torripsamments. Soluble salts are washed into the profile increasing the electrical conductivity between 25-40 cm deep.

In the soils, texture (Boujocos), N (Kjeldahl), P (UV visible spectrophotometry), K (Pratts), Na (flame photometry), Ca and Mg (complexometry with EDTA), pH and electrical conductivity were determined. Samples of soil for analysis were taken from the first 20 cm of the soil, bearing in mind that 91 to 100% of the roots of the *O. ficus indica* are found in the top 15 cm of the ground (27). To know the soil parameters at greater depth, other soil samples were also extracted at 40 cm depth.

O. ellisiana was used in this study due to its resistance to low temperature (12). This experience started in the autumn of 2014. Double cladodes, homogeneous in

size, provided by the IADIZA experimental collection, were used for the plantation. They were cut before they started giving out new cladodes.

Sampling of cladodes took place at the end of the growing period, as from the month of April. Organic matter (OM), dry matter (DM), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest and Wine), crude protein (CP) by Kjeldahl, P (vanadomolibdophosphoric complex colorimetric, Spectronic 21 Bausch & Lomb.), K and Na (flame photometry, Metrolab 315), Ca and Mg (atomic absorption spectroscopy, Perkin Elmer AAnalyst 200) were determined in the cladodes. The samples were obtained from the south and from the north, under the *Prosopis* canopy; a similar sampling being carried out outside the influence of the canopy.

The experimental design included 18 and 23 plants located under and outside of the *Prosopis* canopy, respectively. Under the canopy of five trees, four plants were implanted, two to the north and two to the south, equidistant between the trunk and the edge of the canopy. Five plots with five double cladodes in each of them were also used as controls. Two plants under *Prosopis* spp. and control, respectively, did not thrive in their growth. After a year of the plantation, all the cladodes were collected, with the exception of the double cladodes planted when initiating the experience.

In order to determine the photosynthetically active radiation (PAR), a linear radiometer (Apogee MQ 300) was applied. To quantify the air temperature at 1 m height from the ground and 5 cm deep in the ground, a multichannel digital thermometer was used.

The data for PAR and temperature were registered in spring (September), summer (February) and winter (June), between 11 a.m. and 1 p.m. These reports were taken in the centre of the tree and

outside the influence of the canopy; the orientation being north-south.

The data were analysed using multivariate analysis with InfoStat statistics software. As confirmation, multivariate analysis of variance (MANOVA) of the nutritional variables was performed. To compare mean vectors between the groups, a Hotelling test was used (21).

RESULTS AND DISCUSSION

In each sampling period the air temperature had very similar values both outside and under the canopy. The values of radiation and soil temperature were higher outside the canopy (table 1, page 133). Although the influence of the radiation level on the growth and productivity of *Opuntia* has been demonstrated (1, 9, 11, 14), in this experiment the *Prosopis*' effect on the cactus was more important than the higher radiation received by the cladodes outside the canopy. Shadow of *Prosopis* influenced the incident radiation with a decrease of 20% in summer, 15% in winter and 32% in spring, compared to outside the canopy. During the post-implant and the harvest of the cladodes the temperatures recorded were not low enough to evaluate any damage by frost, having registered -0.7 and 1°C as the coldest days of winter of 2014 and 2015, respectively.

In soil, under *Prosopis* canopy, the contents of N, OM and others elements such as P and K were greater than those found outside it (table 2, page 133).

The contribution of nitrogen by *Prosopis* mulch under its cover could be explained by the redistribution of the nutrients absorbed by the radical system (3, 28) and the biological fixation of nitrogen by *Rhizobium* (15), and in addition, through of the litter accumulated under the canopy.

Table 1. Values of photosynthetically active radiation, air and soil temperature in February, June and September outside and underside of *Prosopis* canopy.

Tabla 1. Valores de radiación fotosintéticamente activa, temperatura del aire y del suelo en febrero, junio y setiembre bajo y fuera de la canopia de *Prosopis*.

	February		June		September	
	Outside	Underside	Outside	Underside	Outside	Underside
PAR ¹ ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	1352	273	775	119	1354	587
AT ² (°C)	31.9	31.4	15.0	14.8	30.4	29.9
TS ³ (°C)	24.5	16.0	5.0	0.0	31.0	19.9

¹Photosynthetically active radiation. / ¹Radiación fotosintéticamente activa.

² Air temperature. / ² Temperatura del aire

³ Soil temperature. / ³ Temperatura del suelo.

Table 2. Soil components at 20 and 40 cm depth outside and under the *Prosopis* canopy.

Tabla 2. Componentes del suelo a 20 y 40 cm de profundidad bajo y fuera de la canopia de *Prosopis*.

	N (ppm)	OM (%)	pH	EC ($\mu\text{S cm}^{-1} 25^\circ\text{C}$)	P (ppm)
20 cm outside	532	1.6	7.1	2650	9.0
20 cm under	1708	3.5	7.4	3130	16.4
40 cm outside	560	2.1	7.5	4960	7.4
40 cm under	798	2.2	7.5	5210	7.9

	K (ppm)	Ca (ppm)	Mg (meq l ⁻¹)	Na (meq l ⁻¹)
20 cm outside	1095	14.5	12.5	3.0
20 cm under	2581	9.2	12.9	2.5
40 cm outside	938	17.8	13.8	27.0
40 cm under	1642	15.9	21.8	12.8

Throughfall effects are generally attributed to leaching from leaves, but canopies also filter and capture atmospheric nutrients, and these processes contribute large amounts nutrients to the understory.

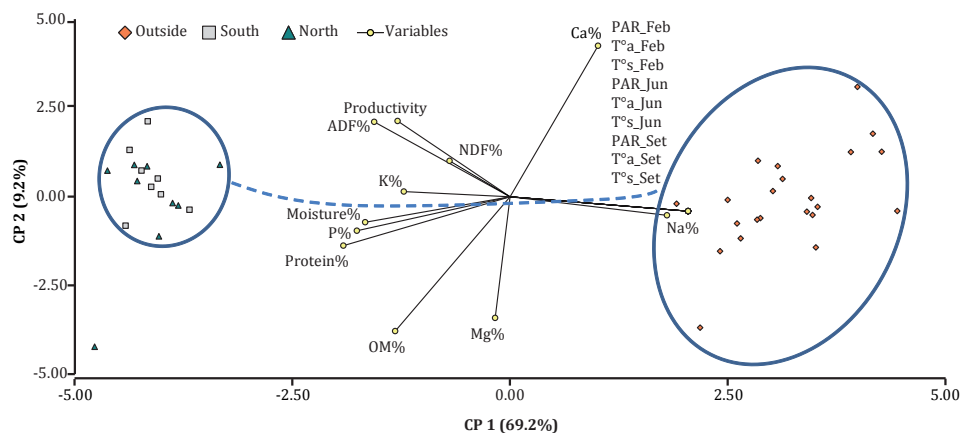
The high percentages of nitrogen, derived from atmosphere in wood and leaves, clearly indicate that *Prosopis* is actively fixing nitrogen (15).

There is evidence that canopy filtration of atmospheric nutrients provided K, Mg, Ca, Na and P (7).

In the principal component analysis (PCA), considering the data obtained from cladodes and soil, the first two axes of variation explain 78.4% of the total variability (figure 1, page 134).

The first component separates cladodes according to the nutrient contents and productivity; to the right, cladodes under the canopy with higher values, to the left cladodes outside the canopy and closely related to environmental variables.

No differences were found in the variables measured for cacti located in the northern and southern parts of the canopy.



T°a: air temperature; T°s: soil temperature; PAR: photosynthetically active radiation; OM: organic matter; ADF: acid detergent fiber; NDF: neutral detergent fiber.

T°a: temperatura del aire; T°s: temperatura del suelo; PAR: radiación fotosintéticamente activa; OM: materia orgánica; ADF: fibra detergente ácida; NDF: fibra detergente neutra.

Figure 1. Biplot obtained by principal component analysis on nutritional and environmental parameters under and outside the *Prosopis* canopy for 41 *Opuntia* plants. Different symbols indicate under/outside location and north/south orientation below the canopy.

Figura 1. Gráfico biplot obtenido por análisis de componentes principales sobre los parámetros nutricionales y ambientales fuera y bajo la canopia de *Prosopis* para 41 plantas de *Opuntia*. Diferentes símbolos indican la ubicación bajo/fuera y la orientación norte/sur debajo de la canopia.

Furthermore, to compare mean vectors between groups and confirm the information given by PCA, a Hotelling test was used.

Table 3 (page 135), presents the bromatological values and cladode quantity per plant of *O. ellisiana* planted outside and under the *Prosopis* canopy.

Dry matter productivity per plant (g) were 34.9 ± 16.1 and 48.1 ± 24.1 outside and under the canopy, respectively. Under the *Prosopis* canopy, the nutritional values of *O. ellisiana* were increased, the nitrogen content, to almost double its value. Productivity cladodes per plant and concentrations of moisture, OM, ADF, NDF, K and P in the cladodes were also signifi-

cantly higher under *Prosopis*, while Ca and Na were higher outside the canopy. Magnesium values were not affected by the position, under or outside the canopy. Under the crown of the tree the effective precipitation is greater than outside of it, due to the runoff of the branches and trunk (20), which favors the leaching of Na in the soil; while an increase in N and P reduces the uptake of Ca and Na (8).

The improvement in the forage value of cactus under the *Prosopis* crown indicates the better condition of the site as a result of the higher nutrient content of the soil and the contribution of OM as mulch, resulting in the formation of fertility islands (26).

Table 3. Means and standard deviations of bromatological values and yield of cladodes per plant of *O. ellisiana* planted outside and under *Prosopis* canopy.

Tabla 3. Medias y desviaciones estándar de valores bromatológicos y producción de cladodios por planta de *O. ellisiana* bajo y fuera de la canopia de *Prosopis*.

Parameters	Outside the canopy (n=23)	Under the canopy		
		North (n=9)	South (n=9)	North and South (n=18)
Moisture (%)	89.1 ±1.3 a	91.9±0.9 b	91.9 ±1.2 b	91.9 ±1.0 b
Organic matter (%)	76.7 ±2.5 a	80.2±2.7 b	79.4 ±1.0 b	79.8 ±2.1 b
Na (%)	0.04±0.01a	0.02±0.01b	0.02 ±0.005b	0.02±0.01b
K (%)	3.1 ±0.4 a	3.5±0.4 b	3.7 ±0.4 b	3.6 ±0.4 b
P (%)	0.07±0.02a	0.11±0.02b	0.12±0.02 b	0.11±0.02b
Ca (%)	5.5 ±1.4 a	4.3±1.2 b	4.7 ±0.4 b	4.4 ±0.9 b
Mg (%)	1.9 ±0.2 a	1.9±0.2 a	1.8 ±0.2 a	1.9 ±0.2 a
Crude protein (%)	4.4 ±1.0 a	8.1±1.3 b	8.0 ±0.5 b	8.1 ±1.0 b
Acid detergent fiber (%)	13.7 ±2.4 a	17.7±1.9 b	18.8±1.0 b	18.2 ±1.6 b
Neutral detergent fiber (%)	29.0 ±4.2 a	31.8±2.4 b	31.2 ±1.3 b	31.5 ±1.9 b
Cladode quantity per plant	3.1 ±1.2 a	5.7±2.7 b	5.6 ±2.1 b	5.7 ±2.3 b

Hotelling test Alfa=0.05. Means with a letter in common are not significantly different (p>0.05).

Prueba de Hotelling Alfa=0,05. Medias con letra en común no son significativamente diferentes (p>0,05).

In arid and semiarid ecosystems, dominant woody plants are likely to cause changes in microclimate and soil properties by mitigating harsh environmental conditions (*e.g.*, high temperature and radiation) and by modifying soil characteristics, resource availability (*e.g.*, water and nutrients) and spatial distribution of nutrients (2, 6, 19).

The nurse effect of *Prosopis* improves the nitrogen content of cladodes in the same way as cattle manure applied to soil (10). The high doses of chemical fertilizers applied almost doubled the CP mean content of the 1-year-old cladodes when it was compared with the treatment in which no fertilizer was added: 7.8 and 4.3% DM, respectively (17).

CONCLUSIONS

There is no significant influence (p>0.05) of the north/south orientation under the canopy on the measured variables.

There are significant differences (p<0.05) in productivity and nutrient content for cactus planted under and outside the *Prosopis* canopy. The nurse effect of *Prosopis* improves the nitrogen content of cladodes in the same way as cattle manure or high doses of chemical fertilizers applied to soil. The enrichment of the pastoral system in arid environments by the introduction of *Opuntia* associated to *Prosopis* increases both the productivity and forage quality of the system.



Sol Curto

Ilustradora

Nombre de la obra: *Prosopis flexuosa* II

Técnica: Grafito sobre papel

Tamaño: 12cm x 12cm

Año 2018

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Influence of interannual rainfall variability on the interception process in a continental mediterranean climate

Influencia de la variabilidad interanual de la lluvia sobre el proceso de interceptación en un clima mediterráneo continental

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ABSTRACT

The interception of rainfall by vegetation greatly affects the hydrology of a basin. The main objective of this paper is to evaluate the effect of annual rainfall variability on the interception in isolated individuals of two of the most common types of vegetation, *Pinus pinea* and *Cistus ladanifer*, in a Mediterranean watershed. For this, experimental data were taken for four years and the Rutter interception model was used to calculate the canopy drying time, while Rutter and Klaassen methodologies were used to determine the storage capacity of the canopy. On the whole, 29.6% of rainfall was intercepted for pine, and 17.7% for rockrose. Stemflow accounted for 0.3% for pine and 17% for rockrose, while 70.1% of the rainfall fell as throughfall for pine and 65.3% for rockrose. However, in dry years, the interception percentage almost doubled that of wet years. An average canopy drying time of 9.2 hours was established for pine, and 4.4 hours for rockrose. Approximately equal results of canopy storage capacity were obtained from both methods (1.8 mm for pine and 0.7 mm for rockrose). Both species registered higher values of canopy storage capacity in dry years than in wet years.

Keywords

Pinus pinea • *Cistus ladanifer* • southern Spain • canopy reservoir capacity • canopy drying time

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RESUMEN

La interceptación de la lluvia por la vegetación afecta sobremanera la hidrología de una cuenca. El objeto de este trabajo es determinar el efecto de la variabilidad anual de la lluvia sobre la interceptación en individuos aislados de *Pinus pinea* y *Cistus ladanifer*, en una cuenca mediterránea. Para ello, se tomaron datos experimentales durante cuatro años y se usó el modelo de interceptación de Rutter para calcular el tiempo de secado del dosel, en tanto que para determinar la capacidad de almacenamiento del dosel se consideraron las metodologías de Rutter y Klaassen. En conjunto, el 29,6% de la lluvia fue interceptada por pino y 17,7% por jara. El 0,3% en pino y el 17% en jara ocurrió como escorrentía cortical, y el 70,1% fue trascolación en pino y el 65,3% en jara. Sin embargo, en años secos, el porcentaje de interceptación casi duplicó el de años húmedos. El tiempo de secado medio en pino fue 9,2 horas y en jara 4,4. Aproximadamente, se obtuvieron iguales resultados de la capacidad de almacenamiento del dosel usando ambos métodos (1,8 mm en pino y 0,7 en jara), registrándose también valores más altos en años secos.

Palabras clave

Pinus pinea • *Cistus ladanifer* • sur de España • capacidad de almacenamiento del dosel • tiempo de secado del dosel

INTRODUCTION

The partitioning of rainfall affects the water balance both locally and at basin scale, and accordingly the structure of the vegetation cover significantly affects the hydrology of a watershed. During and after a rainfall event, the vegetation temporarily stores rainwater on the canopy, which subsequently evaporates (interception), resulting in a reduction in the water available for infiltration (38). Rainfall that is not intercepted, falls as throughfall, affecting the surface layers of the soil and the rate of deep percolation below the root zone in a discontinuous forest cover, while stemflow can reach the deeper layers of soil and make water directly available to the roots. Some studies in the Mediterranean basins indicate that forest cover leads to a significant reduction in average annual measured flows, and so from a hydrological perspective, vegetation canopies exert significant control by modifying

both evaporation and precipitation redistribution (4). It is demonstrated that interception losses represent a significant component of the water balance in forest areas (24). These losses can vary from 9% of incident rainfall in the Amazon rainforest (26) to almost 50% in coniferous forests in areas of Mediterranean mountainous climate (25).

The most common field method of determining interception is to measure incident precipitation, throughfall and stemflow. Interception is then the difference between precipitation and the sum of throughfall and stemflow. In general, trees have a higher relative throughfall (the ratio of throughfall to gross rainfall) and a lower relative stemflow than shrubs (26, 39).

Whatever the method used to obtain the canopy storage parameters for modeling interception, there is no

standard protocol for measuring incident rainfall, throughfall and stemflow in interception studies (21). This is due, on the one hand, to the variety of study objectives and diversity in vegetation coverages studied (22) and, on the other hand, to the fact that sampling systems are not well adapted to the heterogeneity of the forests studied (40). The accuracy of measurement of these variables can give rise to negative interception values or, at least, errors in the interpretation of the measurements obtained (1, 8).

However, the process of interception is influenced by two main factors: the climate conditions and the structure of the species of trees or shrubs (35). In literature, no consensus exists on whether climate or vegetation is the dominant factor. For instance, (3) compared stemflow from *Pinus sylvestris* with five tree species at three sites in northern Britain and found that it was influenced by stand characteristics more than by weather conditions. On the other hand, (12) showed with a modeling study that the rainfall distribution is most dominant for the interception amount.

In Mediterranean forest where canopy cover is discontinuous, the wide variability in the relationships between rainfall and interception losses creates greater complexity (6, 15). In areas of *dehesas* pasture, such as our study area, where the forest is composed of isolated trees interspersed with shrubs, this complexity is even greater (5).

Canopy water storage capacity is a key factor determining the quantity of precipitation intercepted (13, 16). When rainwater that falls on the canopy exceeds the maximum holding capacity water begins to fall to the ground along with the water that manages to pass directly through

the canopy without being intercepted (*i.e.*, free throughfall). These parameters, the canopy water storage capacity (S) and the free throughfall coefficient (p), are related to climatic conditions during rainfall and to the canopy structure (26).

In coniferous forests, there is a close relationship between interception and the amount of water stored in the canopy (20). Moreover, in shrubs the water storage capacity can even at times be comparable to that of tree communities and, despite their short height, in arid and semi-arid environments water loss by evaporation in rainy periods may be similar to losses in more temperate forests (9).

Although several experiments have been carried out in areas generally referred to as Mediterranean climates, few have focused on the subtype and none to the best knowledge on a *dehesa* area, while *dehesa*'s are a common ecosystem or land use type in southwestern Iberian Peninsula. The present study was conducted in isolated individuals of the most common types of vegetation in the Mediterranean "El Cabril" watershed, *Pinus pinea* and *Cistus ladanifer*, with objectives: (i) to estimate the impact of the annual rainfall variability on the process of interception; (ii) to determine of the minimum number of hours required for all water stored in the canopy to evaporate and (iii) to calculate the amount of precipitation needed to reach saturation point in both species. While many studies in Mediterranean climates are of short duration this study encompasses a study period of four-year, which allows us to obtain more consistent results than those typically found in literature.

STUDY SITE AND MATERIALS

Site description

The experimental plots are located on a forest area known as "El Cabril" in the town of Hornachuelos, which is in the west of the province of Córdoba, Spain. "El Cabril" has an area of 11.26 km² and its geographic coordinates are 38°4' N and 5°25' W (figure 1).

The climate of the study area is influenced by its two main geographical features: its elevation (500-700 m a. s. l.) and its relative continentality. It is characterized by a semi-arid continental Mediterranean climate, featuring hot, dry summers and moderately cold winters.

The average annual temperature is 16°C, with the maximum ranging between 37 and 41°C, and the minimum between -1 and 3°C. The average annual rainfall (1990-2013) is 506 mm/year with large variations (CV = 0.49), December being the wettest month (average 73 mm/month)

and July and August the driest months (average 4 mm/month).

The rainfall regime is one of the most characteristic climatic aspects, with the majority of precipitation occurring from October to May.

The reason for studying isolated individuals is the frequent existence of sparse vegetation in the basin. The plant communities are dominated by Mediterranean sclerophyllous scrub (6.1 km²) with extensive thickets of rockrose (*Cistus ladanifer*) accompanied by retama (*Retama sphaerocarpa*), mastic (*Pistacia lentiscus*), flax-leaved daphne (*Daphne gnidium*), lavender (*Lavandula stoechas*) and rosemary (*Rosmarinus officinalis*). A large area at the basin rivers' headwaters (2.6 km²) is covered by plantations of pine (*Pinus pinea*), planted in the early 1970s.



Figure 1. Location of the forest area of the "El Cabril".

Figura 1. Ubicación de la zona forestal de "El Cabril".

The relief of the study area is fairly steep, with an average slope of 30%. Other species such as eucalyptus and quercus are also present in the basin, but they cover a smaller area. The soils are sandy loam texture and relatively shallow with a depth not exceeding 30 cm in most of the basin (29).

Materials

Three experimental plots were established to determine throughfall (T_p) and stemflow (S), two of *Pinus pinea* and one of *Cistus ladanifer*. In each plot, there is only one individual of the corresponding species. The study was conducted over a four-year period from October 2010 to June 2014 in the case of the first pine plot and the rockrose plot. For the second pine plot, experimental data is available from September 2013 until June 2014. The characteristics of these plots are shown in table 1.

The second individual of pine has been chosen with similar dasometric characteristics to the first in order to eliminate the influence of geometry on the results obtained. To do this, it was measured the same variables that other authors chose to quantify the growth of

species due to management strategies (*i.e.* Donoso *et al.*, 2016) or to establish the value of individuals of commercial or ornamental interest (*i.e.* Ponce-Donoso and Vallejos-Barra, 2016). These variables, among others, are the height of the tree, the canopy area projection or the diameter at breast height (table 1).

Two tipping bucket raingauges were installed to record the incident precipitation with an accuracy of 0.2 mm (*Eijkelkamp*). Precipitation data are recorded every 10 minutes on a datalogger (e+® RAIN).

The raingauges are located in two open areas, approximately 20 meters away from the pine plots and the rockrose plot. Also, there is a complete meteorological station at El Cabril watershed.

In both pine plots, a permeable fence (two meters high) was installed to protect the instrumentation inside, delimiting an area of 28.27 m², in both cases.

In the pine plots the volume of throughfall (T_p) is measured by eight standard manual raingauges of 9.3 cm in diameter located under the pines according to cardinal directions and forming two concentric rings.

Table 1. Characteristics of experimental plots.

Tabla 1. Características de las parcelas experimentales.

Plot	Latitude	Longitude	Height (m)	Dbh (m)	Canopy area projection (m ²)	Altitude (m)	Orientation	Slope (%)
<i>Pinus pinea</i> (I)	38.076°N	5.421°O	8.7	0.39	33.1	458.1	Southeast	28.0
<i>Pinus pinea</i> (II)	38.076°N	5.421°O	8.8	0.43	34.1	461.4	Southeast	27.6
<i>Cistus ladanifer</i>	38.087°N	5.427°O	1.9	0.05	0.78	410.3	South	40.0

Dbh: Diameter at breast height.

Dbh: Diámetro a la altura del pecho.

In addition, six troughs consisting of 20 cm wide and 2 m long metal guttering were installed radially just below the canopy within the research plots. Pérez-Arellano *et al.* (2016), found that similar statistical results were obtained with any of these methods.

The guttering was installed at a slope of about 10% to encourage the draining of the collectors. Stemflow (S_f) is measured by an open, flexible tube sealed with silicone around the trunk of the trees, and channeling the water collected to a 12-liter tank.

The guttering and the raingauges were cleaned at least once a month but on occasions they still got blocked.

Furthermore, it was also measured T_f and S_f on a rockrose plant that had representative characteristics for *Cistus ladanifer*, by means of an adaptation of the so-called "interception flow collection box" described by Belmonte and Romero (1998). This device consists of a 0.45 m² impermeable square surface surrounded by an L-shaped perimeter that ensures that the throughfall does not overflow, and that it reaches the drainage point located in one of the corners (figure 2). All throughfall water is collected and channeled to a 25-liter tank.

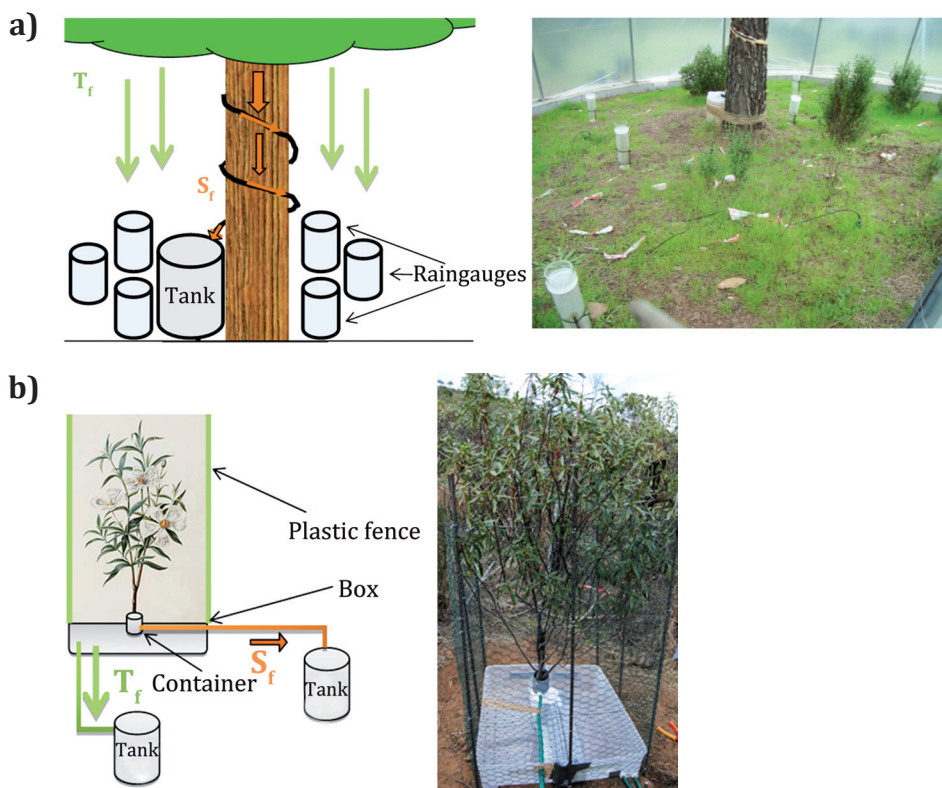


Figure 2. Experimental plots for *Pinus pinea* (a) and *Cistus ladanifer* (b).
Figura 2. Parcelas experimentales en *Pinus pinea* (a) y *Cistus ladanifer* (b).

The plot is also waterproofed laterally to prevent the entry of lateral rain. To measure stemflow, a container was placed around the base of the trunk to collect the water flowing through the branches to the trunk. This water is conveyed to a 12-liter collection tank.

The gaskets of the "interception box", the drain holes and cortical runoff collector were sealed with silicone.

Measurements were taken when the rainfall events had ended and after a sufficient period of time for the canopy storage to empty and for throughfall and stemflow to be fully collected (usually one day after the end of the rain).

Methods

Effect of annual rainfall variability on the process of interception

In this study, the amount of intercepted precipitation has been determined indirectly by taking the difference between the incident rainfall and the measure precipitation passing through the vegetation canopy, which is divided into throughfall and stemflow:

$$I = P - T_f - S_f \quad (1)$$

where:

I = interception (L)

P = incident precipitation (L)

T_f = throughfall (L)

S_f is stemflow (L).

During study period, it was calculated the water balance per precipitation event and we obtained information about T_f and S_f for *Pinus pinea* and *Cistus ladanifer*. The influence of precipitation annual variability on the interception process can be studied because it was disposed of four years of data and a very high number of rainfall events.

Canopy Drying time

A precipitation event is defined as one or multiple showers with at least enough time between the events to dry the canopy, where a shower is a single-rainfall occurrence. In this case, it was applied the Rutter model of interception (35) to estimate canopy drying time, using hourly climatic data from the El Cabril weather station.

The model is based on a calculation of the water balance in the canopy and trunks, and uses incident precipitation and evaporation in the canopy and the trunk to estimate throughfall, stemflow and interception. Wet canopy evaporation can be directly calculated from the Penman-Monteith potential evaporation equation (28).

Pereira *et al.* (2016), have proposed a wet bulb to estimate mean evaporation rate as an alternative to the Penman-Monteith equation when the forest canopy is fully ventilated. The canopy drying time (CDT , h) was obtained by calculating the amount of time it takes for the canopy to dry completely after the end of the rainfall event ("rain shower"); given that we have hourly precipitation and potential evaporation data, we know when the rain stops and how many hours it takes until, according to the model, there is no longer any water stored in the canopy.

Canopy storage capacity

According to Leyton *et al.* (1967), canopy storage capacity (S , mm) can be defined as the amount of precipitation needed to reach saturation point and it can be determined using the graphic method whereby incident precipitation P is plotted against T_f for individual events. Due to the fact that the field readings could comprise a number of rainfall events and that there was no continuous record of throughfall, it was selected only the events with one or two showers to calculate S .

In the case of an event with two showers, the total amount of intercepted water was divided in proportion to the amount of incident precipitation collected in each shower.

The scatter plot demonstrates a two-step relationship between incident precipitation and throughfall (34).

The first step was dictated by canopy storage and the second step occurs after saturation of the canopy.

The intersection point between the regression line fitted to the first-step data and the upper envelope line of the second-step data as proposed by Gash and Morton (1978) and Lloyd *et al.* (1988), represents the event size in which the amount of incident precipitation is sufficient to saturate the canopy (P_s). Finally, the value of S is obtained from the intercept of the upper envelope on the throughfall axis (in absolute value).

Specifically, when the amount of the incident precipitation in an event was less than P_s , the incident precipitation was mostly intercepted by the canopy and the amount reaching the ground, does so as free throughfall; therefore, the regression line slope between T_f and P indicates the proportion of incident precipitation reaching the ground as free throughfall (18). So, for the first step, the coefficient of free throughfall (C), defined as the proportion of incident precipitation reaching the ground as free throughfall, can be calculated as the ratio between T_f and P_s , and the canopy storage capacity can be obtained as suggested (34), by applying equation 2.

$$S = (1 - C) P_s \quad (2)$$

Following the methodology proposed by Huanhua *et al.* (2013), which defines the canopy storage capacity (S , mm) as

the maximum possible water storage after a quick drain has stopped, and calculates S using a scatter plot of measured interception (I) versus P , linear regression of the type $I = \alpha \cdot P + \beta$ is fitted to the second-step data, taking α as the ratio of precipitation that evaporates from the canopy and β as the canopy storage capacity (S).

RESULTS

Effect of annual rainfall variability on the process of interception

It should be noted that in the first and third year precipitation was 60% and 25% higher, respectively, than the average of the previous 20 years, while in the second and fourth year precipitation was 44% and 20%, respectively, below the previous average (table 2, page 147). These data show the interannual variability of rainfall over the years.

It was recorded 64 rainfall events for *Pinus pinea* and 53 for *Cistus ladanifer*. The rain showers had an average intensity of 2.81 mm/hour.

The maximum intensity recorded at 10 and 60 minutes were 13.8 mm and 28.6 mm, respectively.

In *Pinus pinea* the cumulative amount of precipitation measured after 64 events was 2083.6 mm (CV = 0.96), with a high variability (ranging from 1.2 mm to 180.6 mm), being the volume of water intercepted 616.6 mm (29.6%).

In the *Cistus ladanifer* plot, only 53 events could be included in the study due to different problems when taking the samplers (such as filling up the water collection tanks and installation errors). In this case, the accumulated rainfall was 1289.4 mm, with a lower coefficient of variation (CV = 0.74), and an interception amount of 228.6 mm (17.7%).

Table 2. Precipitation and throughfall, stemflow and interception losses for period 2010-2014.

Tabla 2. Precipitación y pérdidas por trascolación, escorrentía cortical e interceptación durante el período 2010-2014.

Period	P (mm)	Tf _{pinus} (%)	Tf _{cistus} (%)	Sf _{pinus} (%)	Sf _{cistus} (%)	I _{pinus} (%)	I _{cistus} (%)
2010-2011	805.7	78.5	70.9	0.4	18.7	21.1	10.4
2011-2012	283.5	61.5	55.5	0.2	16.3	38.3	28.2
2012-2013	635.1	73.6	69	0.4	15.9	26	15
2013-2014	402.4	57.7 (I) 56.4 (II)	63.5	0.1 (I) 0.3 (II)	17.6	42.2 (I) 43.4 (II)	18.9

For *Cistus ladanifer* the average throughfall was 65.3% and the average stemflow was 17%, whereas in *Pinus pinea* these values were 70.1% and 0.3%, respectively. Table 2, shows the results broken down by year. The year 2013-2014 displays results for both the first and second pine.

Canopy drying time

For the pine, the Rutter model identified a total of 278 rainfall events ("rain showers") with an average drying time of 9.2 hours. The canopy drying time for rockrose is 4.4 hours, and 374 rainfall events were identified in this case. Erring on the side of caution, it was established a minimum period of 10 hours to evaporate all the water stored in the canopy for *Pinus pinea* and 5 hours for *Cistus ladanifer*.

Based on the CDT values obtained, it can be assumed that the methodology followed for taking measurements of throughfall and stemflow is appropriate. All water from throughfall and stemflow had already been collected by the different tanks the day immediately after rainfall.

Canopy storage capacity

Figure 3 (page 148) shows the relationship between incident precipitation and throughfall of 38 showers at the first pine plot and of 35 showers at the rockrose plot (see section 3.3). Measurements from the second pine plot are not shown as there are insufficient data to provide significant results. In these cases, P_s and S obtained graphically are 4.2 mm and 1.8 mm for *Pinus pinea*, and 1.4 mm and 0.6 mm for *Cistus ladanifer*, respectively.

The value of C obtained as the values of slopes of the regression equations between throughfall and incident precipitation for the first step in *Pinus pinea* and *Cistus ladanifer* are 0.58 and 0.51, respectively, as indicated in section 3.3. Applying equation 2 (page 146), gives S values of 1.76 mm for *Pinus pinea*, and 0.69 mm for *Cistus ladanifer*. Both values are in close agreement with those obtained graphically (1.8 mm for *Pinus pinea* and 0.6 mm for *Cistus ladanifer*).

These results were compared following the methodology proposed by Klaassen *et al.* (1998), figure 4 (page 149), that yielded values of 1.81 mm for *Pinus pinea*, and 0.74 mm for *Cistus ladanifer*, similar to those obtained above.

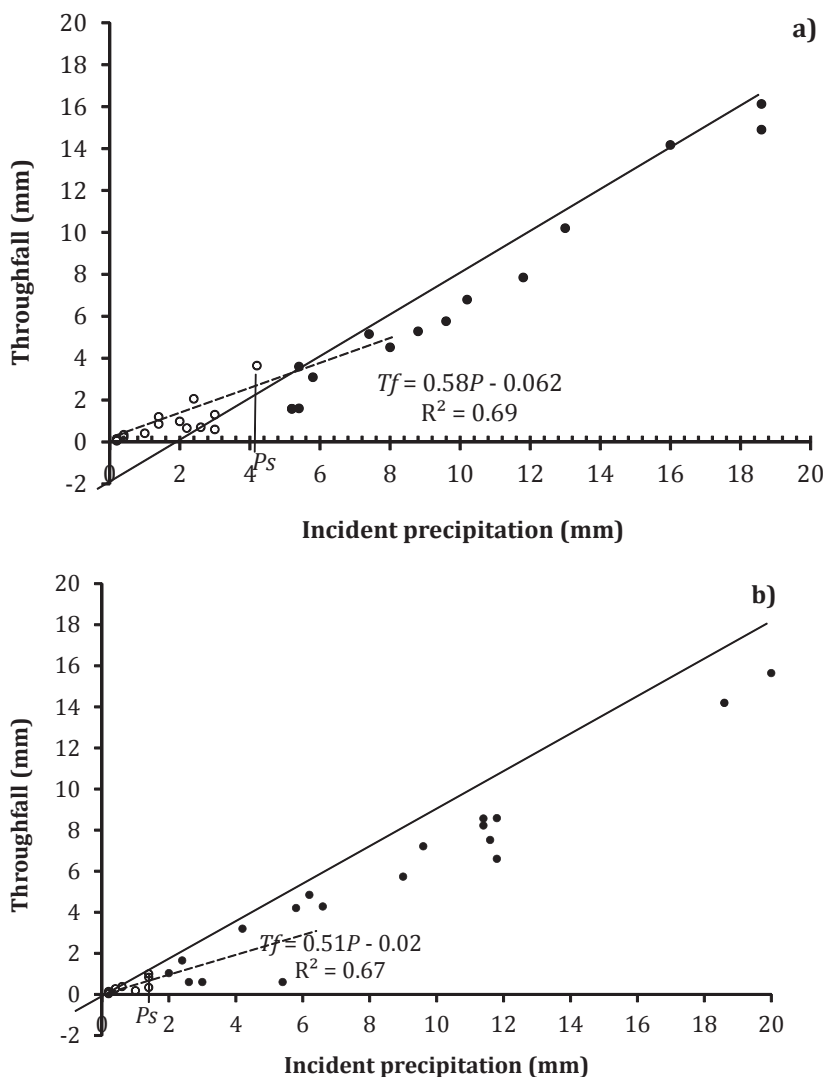


Figure 3. Scatter plot of throughfall against incident precipitation ($Tf > Ps(\circ)$; $Tf < Ps(\bullet)$) for *Pinus pinea* 1 (a) and *Cistus ladanifer* (b). The regression line fitted to the first-step data and the upper envelope line of the second-step data is depicted with a discontinuous and continuous line, respectively (Canopy storage capacity).

Figura 3. Representación de la trascolación frente a la precipitación incidente ($Tf > Ps(\circ)$; $Tf < Ps(\bullet)$) para *Pinus pinea* 1 (a) y *Cistus ladanifer* (b). La línea de regresión ajustada a los datos del primer escalón y la línea envolvente superior de los datos del segundo escalón se representan mediante una línea discontinua y continua, respectivamente (Capacidad de almacenamiento del dosel).

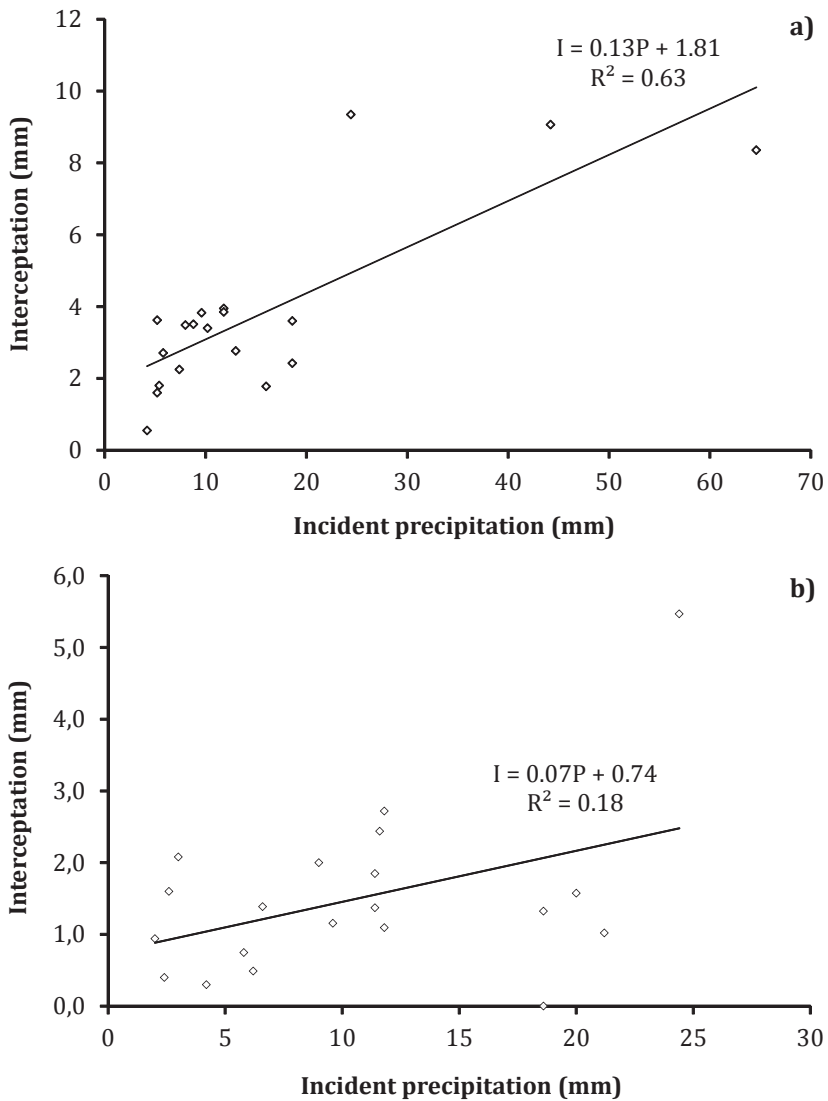


Figure 4. Scatter plots of interception against incident precipitation for *Pinus pinea* I (a) and *Cistus ladanifer* (b).

Figura 4. Representación de la interceptación frente a la precipitación incidente para *Pinus pinea* (a) y *Cistus ladanifer* (b).

The value of S varies between the two types of years being greater in dry years (2.07 in pine and 1.09 in rockrose) than in wet years (1.71 in pine and 0.61 in rockrose). This would seem logical since in dry years the wet canopy evaporation increases and, consequently, the storage capacity of the plant also increases.

DISCUSSION

Effect of annual rainfall variability on the process of interception

The results show that the canopy interception differs per vegetation type and annual precipitation (table 2, page 147).

The interception rate is higher in dry years for both species, and is nearly double that of wet years. This can be explained with reference to the canopy storage capacity, which decreases in rainy years and is exceeded more easily, as will be made clear in section 4.3. Accordingly, throughfall is lower in dry years than in wet years.

The results from both the first and second pine are very similar, which might initially suggest that a single year is enough to record conclusive measurements. Nevertheless, table 2 (page 147), shows widely varying results between dry and wet years, thus indicating that study periods of more than a year are advisable.

There is a significant difference between species in terms of the redistribution of precipitation. Stemflow is negligible in *Pinus pinea*, but substantial in *Cistus ladanifer*. This is largely due to differences in plant structures.

In rockrose, the relative stemflow is very variable, which may be an indication of the effect of the resin produced by these shrubs, that acts as a repellent against the first drops of rain.

García-Estringana *et al.* (2010), showed that the impermeable wax, ladane, secreted by *Cistus ladanifer* acts as a water repellent and diminishes the retention of intercepted rainfall. Plant surfaces are rendered water repellent by three-dimensional microstructures of the epidermal cells and hydrophobic wax crystals (17).

The amount of throughfall for *Cistus ladanifer* is lower than that obtained by Simões *et al.* (2009), in Évora (southern Portugal) for the same specie (79% T_p) during two years, but higher than *Cistus salviifolius* (61% T_p). Such variation may be mostly a result of differences in canopy structure; *Cistus ladanifer* is characterized by sparse foliage clumps distributed vertically and with larger gaps in the canopies.

For *Pinus pinea* the results are consistent with those obtained in two studies carried out in a Mediterranean climate: Llorens *et al.* (1997), for *Pinus sylvestris* (24% I) in Barcelona, Spain; and Ibrahim *et al.* (1982), for *Pinus pinea* (27% I) in Petit Saint-Jean, France.

However, Ghimire *et al.* (2012), in their study of a forest of *Pinus roxburghii* located in Nepal recorded slightly different proportions of throughfall, stemflow and interception, at 83%, 0.5% and 16.5% of precipitation, respectively. This demonstrates the impact of climate on the interception process in similarly-structured species.

Levia Frost (2003), linked the large amounts of stemflow in rockrose, in all cases and even with low-intensity rains, to the plant having a cone-or funnel-shaped structure. *Cistus ladanifer* features just such a structure, with vertically-oriented branches at an insertion angle of more than 45° to the horizontal, thus favoring the flow of water from the branches to the trunk. However, in pine, cortical runoff is lower in drier years because the intensity

and duration of rainfall decreases. Once again, the limited data available for the second pine means that results are of little relevance.

Canopy drying time

Canopy drying time generally varies depending on evaporation rate after the rain (10, 35). There is no standard criterion for the duration of the drying period, as it depends largely on the climate and natural processes affecting precipitation. Some authors have set this time at 4 hours (33), while others estimate 12 hours (3).

This results show that canopy drying time varies from specie to specie, being more than double for *Pinus pinea* than for *Cistus ladanifer*, which makes sense given the difference between canopy structure of both species, that is much greater for *Pinus pinea*.

Canopy storage capacity

The results are consistent with experimental values for interception measured in both plant species. It is indeed the case that a greater amount of incident precipitation is required to saturate the pine canopy compared to the rockrose. Accordingly, the canopy storage capacity is greater for pine.

For small quantities of incident precipitation-too small to saturate the canopy-the slope of the line-of-fit is practically the same in pine and in rockrose (figure 3, page 148), indicating equal throughfall increases in both species in response to equal increases in precipitation. Under these conditions, the only throughfall is free throughfall. Moreover, the slope in each case should show the relationship between throughfall and the incident precipitation, or the coefficient of free throughfall, with a zero intercept. Values different from zero obtained from

said intercept in this study, although relatively small, are due to expected experimental measurement errors.

Thus it can be seen that either method can be used to determine such information, although it is advisable to use both in order to confirm the validity of the results, particularly with low values for the coefficient of determination R^2 , as was the case for *Cistus ladanifer* ($R^2=0.18$) when the Klaasen methodology was used (figure 4, page 149).

These values are similar to those obtained by Saxena (1986), for a pine forest in Uppsala ($S = 2.1$ mm, $C = 0.49$ and $P_s = 4.1$ mm).

For *Cistus ladanifer*, we find a higher value than García-Estringana *et al.* (2010), who obtained an average S value of 0.54 mm using eight individuals in a laboratory setting. The different values obtained by different authors may be due, among other reasons, to the use of short data series that fail to reflect the annual rainfall variability.

The difference between dry and wet years in the value of seems logical since the maximum evaporation rate from wet/saturated forest canopies increases in dry years and, consequently, the storage capacity of the plant increases.

CONCLUSIONS

This research show that the canopy interception differs per vegetation type and annual precipitation. Notable differences in interception values were found when comparing dry and wet years. The interception rate is higher in dry years for both species, and is nearly double that of wet years. Accordingly, throughfall is lower in dry years than in wet years. Then, to reach significant conclusions in interception studies, the use of data

from periods of greater than one year is therefore recommended.

Canopy drying time for each species following a rain event is quite different given the morphology and plant architecture differences between both species, being more than double for *Pinus pinea* than for *Cistus ladanifer*.

A greater amount of incident precipitation is required to saturate the pine canopy compared to the rockrose.

Accordingly, the canopy storage capacity is greater for pine.

The value of canopy storage capacity varies between the two types of years being greater in dry years than in wet years, in both species, because the canopy storage capacity decreases in rainy years and is exceeded more easily.

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Regulación del mercado para conservar suelo agrícola. Interfaz urbano-rural, zona metropolitana de Mendoza, Argentina

Market regulation for conserving agricultural soil. Urban-rural interface, metropolitan area of Mendoza, Argentina

María Elina Gudiño

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RESUMEN

La expansión urbana descontrolada lleva a una fuerte presión especulativa inmobiliaria y consecuentemente a la desaparición de pequeñas explotaciones agrícolas. La atención se centra en la interfaz urbano-rural del área metropolitana de Mendoza, Argentina donde se demuestra que este fenómeno se debe a que los instrumentos normativos aplicados para ordenar los usos del suelo no han logrado regular el mercado del suelo urbano ni conservar suelo agrícola. Se analizan las relaciones competitivas del mercado de suelo, las diferenciaciones que surgen entre los usos del suelo y el rol que cumple el Estado para corregir las imperfecciones y sesgos especulativos. Las aportaciones teóricas de Ciriacy-Wantrup permiten valorar los aportes del Ordenamiento Territorial para planificar y regular usos del suelo mediante la aplicación de instrumentos propuestos en la Ley N° 8051/09 de Ordenamiento Territorial y Usos del Suelo de Mendoza. Si bien los resultados alcanzados no se pueden generalizar, por tratarse de un estudio de caso, se comprueba la aplicabilidad de la metodología a otros casos de similares características y se brindan nuevos aportes a la problemática planteada, contribuyendo a mantener las condiciones de sostenibilidad urbana y rural y mejorar las condiciones de vida de los que habitan en interfaz urbano-rural.

Palabras clave

Usos del suelo • mercado • Estado • zonificación • instrumentos económicos

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ABSTRACT

The uncontrolled urban expansion leads to strong speculative real estate pressure and consequently to the disappearance of small farms. The focus is on the urban-rural interface of the metropolitan area of Mendoza, Argentina, in which it is tried to demonstrate that this phenomenon is due to the normative instruments applied to order the land uses have not been able to regulate the urban land market nor conserve agricultural land. The competitive relationships of the land market and the differentiations that arise between land uses are analyzed and the role of the State is addressed to correct imperfections and speculative biases. The theoretical contributions of Ciriacy-Wantrup make it possible to assess the contributions of Territorial Planning to plan and regulate land uses through the application of instruments proposed in Law N° 8051/09 Territorial Planning and Uses of Soil of Mendoza. Although the results achieved can not be generalized, as it is a case study, the applicability of the methodology to other cases of similar characteristics is verified and new contributions are provided to the problem, contributing to maintaining the conditions of urban and rural sustainability and improve the living conditions of those who live in urban-rural interface.

Keywords

Uses of land • market • State • zoning • economic instruments

INTRODUCCIÓN

La industrialización del siglo XIX trae consigo la concentración de población en grandes ciudades y movimientos migratorios del campo a la ciudad, pero en el Siglo XX y especialmente desde fines de los años '70, el proceso de globalización genera movimientos centrífugos desde las ciudades hacia sus periferias. La ciudad compacta da lugar a una urbanización difusa que lleva a ocupar tierras fértiles que son aprovechadas por pequeñas explotaciones agrícolas.

"Los usos agrícolas de secano son desplazados por los de regadío y estos a su vez desplazados por los urbano-industriales. Ese desplazamiento se produce porque los suelos urbanos-industriales son más competitivos que los agrícolas, pero ante la necesidad de conseguir que las relaciones de competencia no terminen con los suelos de mayor capacidad agronómica, pueden establecerse mecanismos de control y restricción que lo impidan" (44).

La explicación se encuentra en la competencia, forma de comportamiento del mercado que debe ser controlada por el Estado para proteger en iguales condiciones. Sin embargo, sus acciones de intervención no han dado los resultados esperados, lo que plantea la necesidad de buscar nuevas formas de planificación y gestión que permitan entender lo que pasa y actuar conforme a ello. La mirada está puesta actualmente en el Ordenamiento Territorial por considerar lo urbano y lo rural de una manera unificada e integral y sustentar su accionar en la coordinación multinivel de las políticas públicas y la aplicación de instrumentos que permitan regular el mercado de suelo.

Desde esta especialidad y a partir de la selección de un caso de estudio, se pretende demostrar que la zonificación como instrumento normativo utilizado para ordenar los usos del suelo, en el caso

del área de interfaz urbano-rural de la zona metropolitana de Mendoza, no logra regular el mercado del suelo urbano por lo que desaparece el suelo agrícola que bordea la metrópoli.

Se busca analizar el proceso de expansión urbana hacia la zona de interfaz urbano-rural con la consecuente pérdida de explotaciones agrícolas. A través de la interpretación de la forma de comportamiento del sistema productivo de las explotaciones de menos de 5 ha y la selección de criterios que inciden en el precio de mercado, se intenta comprender las relaciones competitivas entre el suelo urbano y el suelo agrícola y el rol que cumple el Estado para corregir las imperfecciones y sesgos especulativos mediante la aplicación de instrumentos que propone el Ordenamiento Territorial en la Ley N°8051/09 de Ordenamiento Territorial y Usos del Suelo de la provincia de Mendoza.

El propósito final es contribuir en la búsqueda de soluciones para frenar el avance urbano y conservar suelo agrícola.

Marco teórico-conceptual

La interfaz urbano-rural

Diversas denominaciones geográficas han intentado dar cuenta de este tipo de espacio: periferia urbana, frontera campo-ciudad, territorios de borde, borde urbano/periurbano, cinturón de especulación inmobiliaria, exurbia, interface o ecotono urbano-rural, etc., términos con los que se ha tratado de caracterizarlo ante la dificultad de conceptualizarlo y delimitarlo (1).

La dicotomía entre lo urbano y lo rural y los procesos que se manifiestan en las áreas de interfaz o transición urbano-rural han sido objeto de debate durante mucho tiempo, sin llegar a una validación general. Teniendo en cuenta que el suelo rural es el que no está funcionalmente integrado a

la trama urbana y que el suelo urbanizado es que el que ha sido completamente antropizado, algunas de las denominaciones más conocidas las que siguen.

Periurbano, aquel espacio donde, partiendo de los bordes de la ciudad, se registra una secuencia de intensidades decrecientes en el uso del suelo: de un periurbano denso "de contacto" a un periurbano cada vez más difuso y distante. Johann Heinrich von Thünen (1826) (3), el precursor de las teorías de localización, determinó en el siglo XIX que alrededor de la ciudad se localizan en anillos diferentes según el tipo de cultivo, el primero dedicado a la horticultura y la producción lechera y en el 5to cordón cultivos más extensivos. García Ramón, Tulla Pujol y Valdovinos Perdices (1995), lo clasifican de otra manera: el espacio urbano propiamente dicho, el espacio periurbano o áreas urbanas discontinuas, el espacio semiurbano (con alternancia de usos), el espacio semirural urbanizado, el espacio rural dominado por la actividad agraria pero con algunas influencias urbanas (como las derivadas de la descentralización industrial) y, por último, el espacio rural marginal.

Otra forma de denominarlo es rururbano, proceso evolutivo que afecta a la periferia de ciertas ciudades. Los primeros en difundir el término rururbano son los franceses G. Bauer y J. M. Roux en 1976, expresión que identifica a las áreas que rodean a las ciudades antiguas donde, la presencia dominante de viviendas unifamiliares, dispersas y aisladas, cohabita con la persistencia de áreas agrícolas y forestales (o naturales). Al respecto "La Geografía se vale de términos híbridos, como el rururbano para procurar comprender y explicar, en este caso, la constitución y los procesos ocurridos en las áreas de expansión de las ciudades.

Esta interface o ecotono está compuesta por una cara interna o periurbano y otra externa o rururbano. Sin embargo, en sus orígenes las definiciones de ambos parecían aludir a lo mismo, empleándose uno u otro como sinónimo" (4).

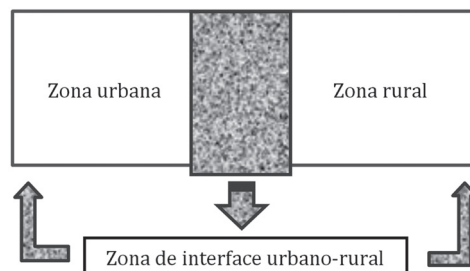
Periurbano y rururbano tienen elementos en común, como la mezcla de funciones y usos del suelo. Sin embargo, se distinguen en el nivel de presión de los usos urbanos sobre los usos de suelo agrícola. En el periurbano, la presión es muy fuerte, debido a la mayor cercanía a la ciudad, de donde provienen las fuerzas centrífugas. En tanto que en el rururbano la presión es menor, debido a una mayor distancia respecto de la ciudad y a una mayor disponibilidad de tierras. Mientras que el periurbano se conformó por el avance de la ciudad hacia su periferia, en el rururbano se dio una combinación de procesos, en distintas escalas temporales y espaciales.

Una connotación diferente tiene el concepto interface, frontera compartida, un enlace entre dos realidades. La Development Planning Unit of University College of London ha condensado tres formas en las que regularmente han identificado aproximaciones a la noción de interface: como periferia de la ciudad, la cual hace referencia a las áreas marginales en proceso de urbanización; como un sistema socioeconómico, es decir, sistemas sociales de pobladores con una condición dual entre lo rural y lo urbano; como la interacción de flujos rurales y urbanos, considerando las áreas periféricas de las ciudades donde se verifican con mayor intensidad vínculos rural-urbanos y son más intensos los cambios y conflictos sociales, ambientales y económicos (figura 1) (30).

La interface es entonces un espacio geográfico donde se manifiestan una serie de conflictos y donde se producen profundas

transformaciones, de carácter sistémico complejo, heterogéneo, delimitable, aunque de difícil inclusión en las zonificaciones de uso del suelo. Un campo relacional en proceso y en continua construcción; un espacio de transición de interacciones de usos del suelo que se caracteriza en esencia por la diversidad de ecosistemas, dinámicas de población y presencia de diversos actores con intereses privados, públicos, comunitarios, no necesariamente convergentes. Un espacio funcional para la ciudad en términos de conservación de la naturaleza, producción de agua y energía, ocio, recreación, seguridad alimentaria y sumidero de residuos.

Un espacio de oportunidades para la expansión de los estilos de vida urbanos y para la conservación de los estilos de vida rurales. Un espacio periférico a la ciudad como consecuencia de procesos de reubicación o relocalización industrial, ligado a la idea de marginalidad económica y social. Un espacio con variedad de ofertas y demandas, en términos de bienes y servicios de carácter ambiental, social y económico y receptor de nueva población debido a procesos migratorios campo-ciudad, provenientes de sectores populares o de población flotante con una condición dual entre lo rural y lo urbano (30).



Fuente: elaboración propia, 2016.

Source: processed, 2016.

Figura 1. Zona de interface urbano-rural.

Figure 1. Area urban-rural interface.

El valor del suelo

El suelo no es neutro, es útil y tiene un valor de uso y de cambio. Según Lefebvre (1973), el espacio juega un papel activo, instrumental y operacional en el conocimiento y la acción del modo de producción capitalista; sirve a la hegemonía, que hace uso de él en el establecimiento, las bases y la lógica del sistema (27). Es además un recurso económico, natural y escaso que tiene distinta valorización según la actividad que haga uso de él y presenta algunas características que contribuyen al aumento de su valor.

En el caso del suelo urbano, es un mercado imperfecto en la competencia, ya que los terrenos difieren entre sí por su forma, subsuelo y superficie, su localización y sus posibilidades constructivas. La oferta de terrenos es inflexible a corto o mediano plazo, mientras que la demanda es rígida en razón de la imperiosa necesidad de habitarlo. Es también un mercado especulativo y complementario del mercado de la construcción.

Entre los componentes del precio del suelo se destacan los gastos para urbanizar un terreno: provisión de agua, evacuación de afluentes servidos, electricidad, servicio de calles, etc., costos que varían además en función de la situación del lote, su facilidad de acceso, su relativa cercanía en relación con otros predios ya urbanizados, etc., lo que exacerba su valor de cambio en relación con el valor de uso (43). Es el que más plusvalías genera y el que aumenta su valor de cambio sobre su valor de uso por lo que cualquier otro aprovechamiento queda supeditado a la dinámica coyuntural del mercado inmobiliario (41).

Con respecto al suelo agrícola este tiene valor de uso y su precio se relaciona con el tamaño de la explotación, la infraestructura, la superficie irrigada, la clase de suelo,

la distancia del predio a la ciudad o ruta más cercana y la zona donde se localiza. Las condiciones de productividad y rentabilidad con las cuales la explotación agrícola compete en el mercado de suelo, dependen de estos componentes.

Con respecto a las relaciones competitivas que se establecen entre el uso del suelo urbano y rural, en la década de los '60 Ciriacy-Wantrup (1964) profesor del Departamento de Economía Agrícola y de los Recursos de la Universidad de Berkeley, realiza interesantes apreciaciones. Destaca que los espacios rurales han sido dejados de lado por la economía a favor del suelo urbano por considerar que el suelo es un recurso natural ilimitado que actúa de soporte a las actividades urbanas, cuyo uso resulta ser más competitivo (5).

Dada la naturaleza del suelo urbano y agrícola y ante el lema de la competitividad que impone la globalización, el suelo urbano tiene mayor capacidad para obtener más rentabilidad en el mercado y esto se debe a que su productividad es mayor que la del suelo agrícola, aunque no siempre la alta productividad se traduzca en mayor competitividad.

Frente a esta realidad y las características de un mercado de suelo que no es perfectamente competitivo, las administraciones públicas pueden asumir un papel protagonista con el objeto de corregir las imperfecciones y sesgos especulativos para sostener a la agricultura periurbana debido a que, tal como sostiene Ciriacy-Wantrup (1964), el suelo es un recurso limitado, por lo que aquellos con mayor capacidad agronómica deben ser conservados para uso agrícola, decisión que implica una reorientación de la expansión urbana y un costo económico y social que deber ser asumido como una prima de seguro ante una posible pérdida cuantificable económicamente.

El rol del mercado y el Estado en el mercado de suelo

De acuerdo con la concepción del liberalismo, los agentes privados son los que regulan el mercado a través del sistema de precios, asignando a cada cosa el valor que realmente tiene. Sin embargo, la lógica de la acumulación privada puede tener consecuencias sociales nefastas porque no contabiliza la destrucción de valores de uso ni el valor de algunos recursos, motivo por el cual el Estado debe intervenir debido a que su función es cuidar los intereses públicos y satisfacer las necesidades colectivas. Pero su actuación en el marco de la aceleración de los procesos de integración a la economía mundial, no es eficiente.

"La gestión sigue siendo verticalista, compartimentada y rígida, utiliza instrumentos que no responden a las exigencias actuales, además de actuar a destiempo y trabajar en la coyuntura sin tener en cuenta el mediano y largo plazo" (17).

Un instrumento técnico y legal utilizado para ordenar los usos del suelo ha sido la zonificación, la que ha pasado a ser solo una operación que se realiza sobre un plano al responder a una concepción rígida propia de una época cuando el Estado tenía mayor poder para controlar (18).

Con el propósito de intervenir en forma más eficiente en el territorio, el Ordenamiento Territorial plantea una nueva manera de concebir este instrumento, no ya como una delimitación de áreas según usos predominantes sino teniendo en cuenta la aptitud para el uso del suelo, su capacidad de carga, la coexistencia de usos compatibles, su complementación con instrumentos económicos que permitan promover determinadas actividades, conservar otras o regular usos del suelo incompatibles.

Con respecto a este tema, Ciriacy-Wantrup (1964) considera la existencia de tres niveles en el sistema de decisiones: el operativo (productor, organismos públicos,

empresarios, etc.), el institucional o normativo y el político, siendo este último el que puede reorientar los objetivos políticos en relación con la planificación y gestión. Aclara que es necesario un cambio de enfoque en la planificación tradicional, la que solo plantea el modelo de crecimiento urbano, el urban sprawl, siendo necesario un tipo de planificación que unifique la planificación urbana con la planificación de la agricultura, la silvicultura, el agua y el resto de recursos naturales (5).

Las afirmaciones de este autor fundamentan la necesidad del Ordenamiento Territorial como forma de planificación que tiene en cuenta la organización y uso del territorio como una unidad integral y como forma de gestión que contempla instrumentos que permiten corregir las imperfecciones y sesgos especulativos del mercado de suelo, tal como lo propone la Ley N°8051/09 de Ordenamiento Territorial y Usos del Suelo de la provincia de Mendoza. Esta normativa contiene una serie de instrumentos espaciales, entre ellos la zonificación que asociada a instrumentos económicos tales, como la prestación de servicios ambientales, incentivos fiscales, impuestos inmobiliarios diferenciados, etc., pueden contribuir a la conservación de suelos agrícolas en la zona de interface urbano-rural del área metropolitana de Mendoza (29).

MATERIALES Y MÉTODO

Las consideraciones teóricas realizadas sobre la complejidad de las áreas de interfaz urbano-rural y el rol que cumplen el mercado y el Estado en las relaciones competitivas entre el uso del suelo urbano y rural, fundamentan la necesidad de utilizar el estudio de caso y el enfoque de los sistemas complejos para el abordaje de la interfaz urbano-rural de la zona metropolitana de Mendoza.

Se elige el estudio de caso por ser un diseño de fuerte orientación empírica y descriptiva que implican la captación holística de un caso según criterios teóricos seleccionados y la interpretación de las complejidades del mismo en su unicidad, en su particularidad, explicando cómo suceden los hechos y por qué, cuáles son las razones inmediatas y el contexto donde tienen lugar (28).

En primera instancia, se delimita la zona objeto de transformaciones producidas ante la expansión urbana hacia tierras agrícolas, mediante análisis espacial de imágenes satelitales del año 2010 y 2014 y el uso de un sistema de información geográfica (SIG). Las variables seleccionadas son: densidad de población por superficie de radio-fracción censal del año 2010 y delimitación del continuo urbano (zona construida).

Una vez delimitada la zona de estudio y teniendo en cuenta que "un sistema complejo adaptativo (...) es un sistema en el cual los procesos que determinan su funcionamiento son el resultado de la confluencia de múltiples factores que interactúan de tal manera que el sistema no es descomponible" (11).

Se buscan las causas que llevan a la desaparición de las explotaciones agrícolas, a través del análisis del proceso por el que transita la actividad agrícola en el período 2002-2008 y teniendo en cuenta los siguientes componentes del sistema productivo agrícola: tamaño y superficie de las explotaciones agrícolas, tipo de cultivos, forma de tenencia de la tierra, cantidad y el tamaño de las empresas rurales (7, 12, 13, 14, 15, 23, 24, 26, 37).

Es importante aclarar que se utilizan datos cuantitativos como complemento estadístico porque el fin último es la búsqueda de significación y no de

distribución de los datos porque tal como sostienen Neiman y Quaranta (2007), la validez externa (generalización estadística) no es posible en un estudio de caso, ni es un objetivo de este estilo de investigación. Es decir que este estudio de caso no se rige por la lógica estadística de representatividad de la muestra (34).

Los resultados obtenidos llevan a profundizar en el rol del mercado y el Estado en cuanto a las relaciones competitivas del suelo urbano y el agrícola.

Se analizan precios de mercado teniendo en cuenta, los siguientes criterios para el caso del suelo urbano: a) tipo de lote urbanizado y sin construcción en barrios tradicionales (son aquellos que fueron diseñados por el Estado a partir de operatorias de vivienda o por iniciativa privada conforme a lo que determina la Ley de Loteos), b) cerrados o semi-cerrados y privados y c) proximidad a rutas de acceso a la ciudad o al tejido urbano consolidado. Estos datos son obtenidos de artículos periodísticos, relevamientos de usos del suelo, como también de documentos sobre zonificación municipal vigentes y realización de entrevistas a inmobiliarias y talleres con técnicos municipales (36).

En el caso del precio o valor del suelo agrícola, se diferencian explotaciones según su localización en los oasis o en tierras no irrigadas; la disponibilidad del recurso agua (si poseen derecho de agua e infraestructura de riego) y el tipo de cultivo (perennes o anuales).

El rol de Estado se define en función de los instrumentos aplicados para regular los usos del suelo, tanto a nivel del gobierno central como el municipal, utilizando como fuente de información el diagnóstico realizado para desarrollar el ante-proyecto de ley de Ordenamiento Territorial para Mendoza entre los años 2006 y 2009.

El rigor científico con el que se trabaja el estudio de caso permite la aplicabilidad de la metodología o resultados a otros casos de similares características porque "si bien queda claro que no se pueden realizar inferencias generalizantes, los resultados alcanzados pueden ser transferidos a otros casos en un contexto similar" (31).

RESULTADOS

La metrópolis de Mendoza

El área metropolitana de Mendoza se localiza en el oasis norte, uno de los tres oasis que posee la provincia de Mendoza gracias al riego. Cabe aclarar que los oasis de Mendoza son oasis de riego creados por el hombre para hacer frente a las condiciones de extrema aridez del lugar. Estos representan el 3% del territorio de los cuales el 0,25% corresponde a las zonas urbanas donde habita el 79% de la población, concentrándose más del 50% en el área metropolitana en tan solo el 0,16% de la superficie.

El resto de la población (21%) se localiza en forma dispersa ya sea dentro de los oasis o en tierras no irrigadas de llanura o montaña, las que comprenden al 97% de la superficie (figura 2, pág. 163).

Es el aglomerado urbano más importante del oeste Argentino y ocupa un lugar estratégico en los circuitos comerciales del MERCOSUR con el mercado mundial, debido al flujo de mercaderías que circula desde el Atlántico hacia el Pacífico, lo que ha favorecido la llegada de la inversión directa extranjera y de capitales regionales y nacionales. Mientras que los otros centros urbanos de la provincia tienden a perder peso relativo en el concierto provincial, la población del área metropolitana sigue creciendo por lo que aumentan las condiciones

de vulnerabilidad y el deterioro de las condiciones de vida.

Este fenómeno de macrocefalia muestra una dinámica diferente a partir del Siglo XXI. Comienza un proceso de verticalización en la ciudad y expansión descontrolada hacia las zonas periféricas de los municipios que la rodean, preferentemente en Luján de Cuyo, Maipú, Las Heras y Guaymallén.

La población del microcentro migra y esto se debe en parte al cambio de usos del suelo, las residencias dan paso a comercios y la prestación de servicios, a lo que se suma la aparición de importantes hoteles de cuatro y cinco estrellas, hoteles boutique, edificios para oficinas y residencia y grandes centros comerciales. Se genera mayor demanda de espacios, fricciones y competencia desenfrenada por el uso del suelo, problemas de contaminación por ruidos, polución atmosférica, producción de mayor cantidad de basura y de desagües que afectan la calidad del agua superficial y subterránea.

Se incrementa el tiempo que implica el traslado desde los lugares de residencia a los del trabajo. Los medios de transporte adolecen de serias deficiencias por lo que el automóvil pasa a ser una necesidad ineludible, pero no todos tienen acceso a él, lo que contribuye a acentuar las desigualdades.

En consecuencia, los ingresos a la ciudad resultan muy dificultosos debido al incremento del parque automotor y la falta de planificación del transporte, las demoras son importantes y se generan nuevas externalidades negativas, entre ellas el aumento de la contaminación por combustión y ruido.

En la periferia, coexisten barrios cerrados de clase alta o media-alta con villas inestables y barrios populares, en zonas carentes de servicios, equipamientos e infraestructura.

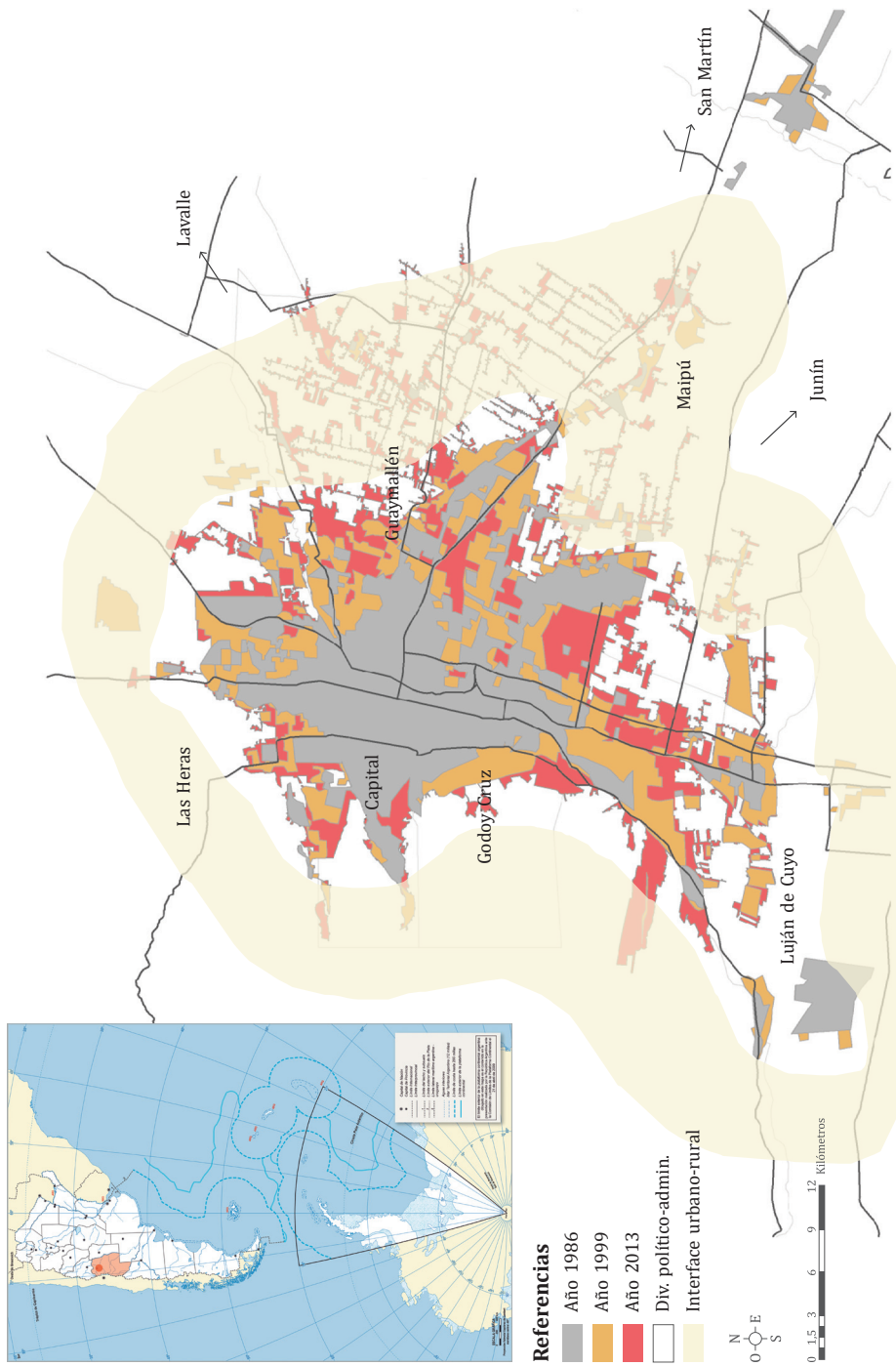


Figura 2. Interfaz urbano-rural, zona metropolitana de Mendoza, Argentina.
Figure 2. Urban-rural interface, metropolitan area of Mendoza, Argentina.

La marginalidad y la segregación social generan violencia y llevan a condiciones de inseguridad. Pero también la inseguridad es producto de habitar en un lugar donde aparecen procesos de degradación natural y riesgos para la población frente a amenazas naturales que pueden terminar en catástrofe. A esto se suma la pérdida de zonas muy fértiles y de alto valor agrícola con la consecuente pérdida de explotaciones agrícolas que amenaza su ecosistema (42).

Expansión urbana y desaparición de explotaciones agrícolas

El mercado inmobiliario orienta la expansión hacia la zona periurbana o interface urbano-rural, en algunos casos hacia el piedemonte y en otros hacia tierras agrícolas localizadas en el cinturón frutihortícola que abastece a la ciudad o en zonas productoras de uvas de alta calidad (figura 2, pág. 163).

El aumento poblacional se produce gracias a la reactivación de la inversión inmobiliaria producida a partir del año 2002, llegando a ocupar el área urbana 26.468 hectáreas en el año 2010, cifra que asciende a 28.574 en el año 2014, según datos del Instituto CIFO, FFyL, UNCuyo (2014).

Este fenómeno explica la disminución de más de 100 explotaciones agropecuarias (EAPs) en Luján de Cuyo y más de 300 en Maipú y cerca de 480 en Guaymallén en el período 2002-2008 (tabla 1).

En total en el período 2002-2008 se pierden en la zona de interface 1041 explotaciones, lo que representa el 35,76% del total provincial. Este proceso continúa y en el año 2010 se urbanizan 11.322 ha de parcelas con derecho a riego (lo que equivaldría a 3.774 EAPs de 3 ha) principalmente en suelos aptos de clase 1 a 3 (muy buenos) según datos del Gobierno de Mendoza (2014). En los

últimos años, los mayores avances de la urbanización, de acuerdo con datos del Departamento General de Irrigación (2015) (38), se producen nuevamente en Luján de Cuyo (2889 ha), Guaymallén (1817 ha), Maipú (1459 ha) y también en Las Heras (1362 ha) mientras que en el resto de los departamentos el crecimiento solo ha sido de menos de 500 ha, excepto en San Martín con 1326 ha, ubicado en la zona Este.

Según el estudio realizado por María Eugenia Van den Bosch en el año 2016, en los distritos de Luján de Cuyo al norte del río Mendoza: Russell, Luzuriaga, Lunlunta, Gutiérrez y Cruz de Piedra en Maipú y todos los distritos bajo riego de Las Heras excepto Uspallata, y de acuerdo con datos de los Censos Nacionales Agropecuarios (CNA) de 1988, 2002 y 2008 (22, 23, 24), se perdieron el 55% de las explotaciones, es decir que solo conservan el 45% de las unidades productivas y el 75% de la superficie.

Tabla 1. Explotaciones Agropecuarias (EAPs) menores a 5 ha, años 2002-2008.

Table 1. Agricultural farms less than 5 ha, years 2002-2008.

Departamento	2002	2008	Variación absoluta
Capital	1	0	-1
Godoy Cruz	0	1	1
Guaymallén	1.213	734	-479
Las Heras	254	157	-97
Luján de Cuyo	387	240	-147
Maipú	2.005	1.688	-317
Total	11.600	8.689	-2.911

Fuente: Instituto CIFO, FFyL, UNCuyo sobre la base de los datos del Censo Nacional Agropecuario, 2002 y datos provisionarios del Censo Nacional Agropecuario-2008 (23, 24).

Source: Institute CIFO, FFyL, UNCuyo based on data from the National Agricultural Census, 2002 and provisional data from the National Agricultural Census 2008 (23, 24).

Fenómeno que se explica fundamentalmente por la presión urbana, lo que le confiere al sector una dinámica muy regresiva y anticíclica en ocupación. Este retroceso afecta en forma muy aguda a explotaciones muy pequeñas y pequeñas, las que representan el 59% de las explotaciones y ocupan el 11% de la superficie, siendo las chacras del sistema hortícola las que más han reducido su presencia tanto en frecuencia como en extensión (7).

La mayor pérdida absoluta de EAP se observa en las propiedades capitalizadas con mano de obra no familiar remunerada. De esta modalidad persiste solamente un 16% de las presentes 20 años atrás. Las unidades productivas de mayores dimensiones se ubican en Vistalba, Las Compuertas, Lunlunta y Russell, distritos más alejados del AMM, frecuentemente sedes de importantes firmas que consignan su superficie en el mismo pero que en realidad se localizan en otra zona (40).

Valor del suelo urbano y suelo agrícola

El suelo tiene distinta valorización según se trate de suelo urbano o agrícola. Con respecto al suelo urbano se consultan precios del m² de suelo urbano en distintos departamentos y distritos del área de interface. La búsqueda se realiza por internet cotejando valores de distintas inmobiliarias, según tipo de lote y proximidad a rutas de acceso. De esta manera se comprueba que no existen variaciones en el precio según el tipo de barrio, por el contrario, en algunos lotes ubicados en barrios tradicionales se encontraron valores superiores a lotes en barrios cerrados o semi-cerrados o privados. Las diferencias no son significativas (6, 9, 20, 21, 32, 33, 35, 39).

Los mayores valores aparecen en Luján de Cuyo, siguiéndole en orden de importancia Maipú y Guaymallén, lugares hacia donde el avance de la urbanización ha sido mayor, mientras que los menores valores corresponden a Las Heras, municipio ubicado en la zona norte del área metropolitana. También se observan variaciones por distritos porque los mayores precios aparecen en aquellos lugares próximos al tejido urbano consolidado, tal es el caso por ejemplo de Mayor Drumond en Luján de Cuyo y el Challao en Las Heras o en aquellos próximos a rutas de acceso a la ciudad como Km11 en Guaymallén, el distrito de Ciudad en Maipú o Chacras de Coria en Luján de Cuyo (tabla 2).

Tabla 2. Valores del suelo urbano y suelo agrícola por distrito, años 2015-2016.

Table 2. Urban land and agricultural land values by district, years 2015-2016.

Departamentos y distritos	Suelo urbanizado 1 m ² -2016 u\$s	Suelo agrícola 1ha- 2015/2016 u\$s
Maipú		15.000-40.000
Fray L. Beltrán	43	
San Roque	45	
Ciudad	60	
Luján de Cuyo		15.000-40.000
Perdriel	57	
Chacras de Coria	79	
Mayor Drumond	60	
Guaymallén		16.000-10.000
Km 11	66	
La Primavera	33	
Corralitos	40	
Las Heras		10.000-8.000
El Challao	63	
El Algarrobal	26	
El Borbollón	45	

Fuente / Source: (32).

Con respecto al valor del suelo agrícola, los valores varían considerablemente según los criterios que se tengan en cuenta.

De acuerdo con datos provistos por inmobiliarias de Mendoza, en el año 2013 una hectárea de tierra explotada en Tupungato, (municipio ubicado en el oasis centro, Valle de Uco), en ese momento la de mayor valor en el mercado debido a la expansión de los viñedos en tierras no irrigadas pero con factibilidad de explotación del agua subterránea y donde crece la compra de tierras de la mano de la inversión directa extranjera, llegaba a u\$s 65.000 por ha. Mientras que en zona no irrigada dedicada a la ganadería era de u\$s 500 y en tierra inculca sin riego de u\$s 30 la hectárea (6, 9, 20, 21, 32, 33, 35, 39).

En el caso de la zona de interfaz del área metropolitana ubicada en zona de oasis y con derecho a riego, el valor del suelo agrícola no solo varía según el tipo de cultivo sino de acuerdo con el tamaño de la explotación, su productividad y rentabilidad.

En zonas como Maipú y Luján las cuales se destacan por su aptitud para cultivos de viñedos, en la década pasada el crecimiento urbano hacia la zona norte de Luján, especialmente Chacras de Coria y Vistalba, generó la pérdida de 700 hectáreas de viñedos y tierra fértil para el cultivo, mientras que en otros lugares de Maipú y

Guaymallén, especialmente en los distritos de Corralitos y La Primavera, el cinturón verde perdió entre el año 2004 y 2014 casi el 10% de su superficie por el avance de la urbanización. Se trata de productores minifundistas, el 68% de las EAPs son menores a 5 hectáreas, porcentaje que llega al 84% si se consideran las EAPs de hasta 10 hectáreas, con alta dependencia de mano de obra familiar, descapitalizados y con maquinarias obsoletas. Más del 40% tienen problemas de tenencia de tierra por lo que pagan altos costos de arrendamiento o cultivan mediante régimen de mediería y aparcería (16) (tabla 3).

"Las unidades pequeñas son el resultado de un proceso histórico de fraccionamiento sucesorio y puede interpretarse su antigüedad, en función de relaciones de proximidad al área urbana de acuerdo con el patrón de crecimiento del oasis y son asimismo, los terrenos más demandados por el crecimiento urbano, lo cual genera un incremento del valor de los terrenos. Esto significa en términos económicos un aumento de los costos de producción por el costo de oportunidad de la tierra y si bien, los mismos no se registran financieramente, juegan un papel determinante en las decisiones productivas de los actores.

Tabla 3. Valor de la tierra en Mendoza, año 2014.

Table 3. Value of land in Mendoza, year 2014.

Localidades	Tupungato = 100	Tierra Inculca U\$S x ha	Tierra Explotada U\$S x ha
Tupungato	100	40.000	65.000
Maipú / Agrelo	80	32.000	52.000
Luján / Perdriel	75	30.000	48.000
Alta montaña/arroyo	1.5	600	n/c
Ganadería extensiva	1.3	n/c	500
Zona no irrigada	0.075	30	n/c

Fuente: Gobierno de Mendoza, Programa de Fortalecimiento Institucional de la Subsecretaría de Planificación Territorial de la Inversión Pública - Préstamo CAF 7353, 2014.

Source: Government of Mendoza, Institutional Strengthening Program of the Secretariat of Planning Territorial Public Investment - Loan CAF 7353, 2014.

Este fenómeno se agudiza en las unidades con menor inversión productiva y de fácil relocalización como son las chacras hortícolas, que fueron las que más perdieron ocupación territorial. La baja escala, frecuentemente por debajo de la unidad económica, reduce las expectativas y por lo tanto inversiones" (40).

Instrumentos de regulación para conservar suelo agrícola

De acuerdo con los estudios realizados para elaborar el ante-proyecto de ley de Ordenamiento Territorial entre el año 2006 y 2009 en Mendoza, los instrumentos normativos para regular los usos del suelo son de competencia municipal y deben ser aprobados por ordenanza.

Cada municipio determina su límite urbano y los criterios de clasificación y zonificación de usos del suelo, lo que genera en el área metropolitana conflictos e incompatibilidades a nivel interjurisdiccional al no existir una delimitación uniforme de la zona de interface urbano-rural, como tampoco criterios comunes acerca de la denominación de las áreas de expansión urbana (la mayoría las denominan zonas complementarias o de reserva) dada la multiplicidad de funciones de la zona de interfaz y la especificidad de usos del suelo rural, especialmente el agrícola.

Es importante mencionar que la Administración Tributaria Mendoza (ATM), es el órgano del gobierno provincial que calcula el avalúo fiscal, ponderación atribuida a la utilidad de un inmueble como bien económico, pero la clasificación y delimitación que utiliza, no coincide con las zonificaciones municipales.

Tampoco existe una relación directa entre el avalúo fiscal y el valor del inmueble en el mercado, lo que influye en la competencia inequitativa de los usos del suelo. El avalúo fiscal es la tasación no comercial

que el fisco realiza sobre un predio o bien raíz, con el fin de valorizarlos y aplicarles una contribución o impuesto territorial. Generalmente el avalúo fiscal es inferior al avalúo comercial. El objeto del avalúo es el inmueble, para ATM, es el terreno más las mejoras, lo que implica considerar además de la superficie de la parcela y superficie cubiertas, la categorización del inmueble (según características constructivas), los usos del suelo y en zonas rurales los derechos de riego.

En función del planteo que Ciriacy-Wantrup (1964) hace con respecto a los niveles de decisión para conseguir la conservación del suelo agrícola, se trabaja con el segundo nivel, el institucional normativo y específicamente en la Ley N° 8051/09 de Ordenamiento Territorial y Usos del Suelo de la provincia de Mendoza, por ser la que puede provocar un cambio en los objetivos políticos al considerar de una manera integral la planificación urbana y rural.

Esta Ley prevé la elaboración de planes de Ordenamiento Territorial jerarquizados según competencias delegadas en la constitución provincial y la incorporación de nuevos criterios de zonificación de usos del suelo asociados con instrumentos económicos que pueden utilizarse para frenar el crecimiento urbano en tierras agrícolas.

El propósito es promover, conservar o desalentar actividades en el territorio según criterios de equidad y sostenibilidad. Sobre la base de esta normativa se elabora en el año 2014 una propuesta de Plan Provincial de Ordenamiento Territorial (36), la que incluye criterios generales y uniformes para todos los municipios en lo concerniente a la zonificación de usos del suelo especialmente en las áreas de interfaz, tales como: la multifuncionalidad, es decir la presencia de espacios con vocación rural, industrial

o de servicios especiales que por sus dimensiones no pueden estar dentro de la unidad urbana; la diversidad ya que pueden convivir áreas residenciales con otros usos compatibles siempre y cuando no generen conflictos ambientales; el tamaño de las parcelas, las que no pueden ser inferiores a una unidad económica de explotación extensiva o intensiva en áreas rurales. Estos criterios se complementan con instrumentos económicos como son la adecuación del impuesto inmobiliario al valor del suelo vigente, sea urbano o rural y el reconocimiento de la prestación de servicios ambientales de las explotaciones agrícolas (19).

La adecuación del impuesto inmobiliario, al valor del suelo vigente, exige mecanismos periódicos de actualización y del estado de mejoras edilicias para que refleje el valor referencial de mercado. Si bien la alícuota es determinada anualmente mediante la Ley Impositiva, se plantea la necesidad de incluir criterios vinculados con la zonificación de usos del suelo para el cálculo del valor del suelo y especialmente considerar el valor del suelo agrícola.

Con respecto a la determinación de la Unidad Económica Mínima (UEM), la Ley N° 8051, en su artículo 21 establece la "reglamentación y elaboración de las normas referidas a la implementación de la Unidad Económica -UE- de acuerdo con las previsiones de los artículos Nos. 2326 y 3475 del Código Civil" para fundamentar la importancia de su conservación. Si bien su estimación resulta compleja, su determinación es fundamental para la preservación de explotaciones agrícolas menores a 5 ha. La estimación de la Unidad Económica Mínima (UEM) es difícil debido a las condiciones de fragilidad ambiental que caracterizan al territorio de la provincia, la diversidad de actividades

productivas que en ella se desarrollan, la concentración de las actividades productivas y económicas en los oasis, la existencia de explotaciones agrarias trabajadas en forma mixta y los precios de la producción que varían notablemente en tiempo y espacio (25).

En relación con la compensación por servicios ambientales se considera la necesidad de cuantificar el coste de la pérdida de suelo agrícola, tal como Ciriacy-Wantrup (1964) propone para los recursos naturales. Esta compensación o subsidio a las explotaciones agrícolas en zonas de interfaz urbano-rural ayudaría a evitar el fraccionamiento en unidades menores y a conservar el uso agrícola, dado que su sustentabilidad se apoya en la necesidad de preservar la soberanía alimentaria de los habitantes de la ciudad y mantener una agricultura de cercanías que contribuya a acortar las distancias entre los habitantes del campo y la ciudad (5).

CONCLUSIONES

La zona de interfaz del área metropolitana de Mendoza es un caso de estudio sobre los problemas que plantea una expansión urbana sin control hacia tierras fértiles agrícolas, un ejemplo de los espacios que circundan las ciudades, los que han sido objeto de profundas transformaciones territoriales y conflictos que plantea la fuerte presión especulativa inmobiliaria.

Se trata de un espacio geográfico sistémico complejo, heterogéneo; de transición e interacciones de usos del suelo, dinámico y diverso. Estas características se expresan también en el comportamiento del mercado de suelo, recurso económico, natural y escaso que adquiere distinta valorización según la actividad que haga uso de él.

Es por este motivo que se centra la atención en las relaciones del mercado y el Estado en cuanto a la valorización del suelo y las diferenciaciones que surgen entre el uso del suelo urbano y el rural, por ser el suelo urbano más competitivo y generador de más plusvalía, lo que aumenta el valor de cambio sobre el valor de uso, mientras que el suelo agrícola es menos competitivo porque sus condiciones de productividad y rentabilidad inciden en el valor de uso. Esta realidad plantea la necesidad de intervención por parte del Estado para corregir las distorsiones que se generan.

Se analiza el estudio de caso como un sistema complejo donde se producen transformaciones territoriales debido al avance de la urbanización sobre suelo rural. Se aborda el sistema productivo de las pequeñas explotaciones que desaparecen, los valores del mercado de suelo que demuestran que las relaciones competitivas entre lo urbano y rural no son equitativas y se plantea la necesidad de aplicación de instrumentos de regulación ante la desigual competencia en el mercado.

Se trata de explotaciones de menos de 5 ha que pierden productividad y rentabilidad, motivo por el cual son abandonadas y posteriormente urbanizadas, pérdida en la que influyen distintos factores vinculados con las características que presenta cada lugar, los usos del suelo que practican, la existencia o no de derecho a riego e infraestructuras, como también las normativas que regulan el uso del suelo y las instituciones involucradas en la gestión del territorio. Por el contrario, las variaciones en el precio del suelo urbano se producen según las zonas donde estos se ubican, apareciendo los mayores precios en aquellos lugares próximos al tejido urbano consolidado de la zona sur y suroeste de la interfaz. Es decir, mientras aumenta el valor inmobiliario del

suelo debido a su cercanía a la ciudad o a vías de acceso rápido, disminuye la renta agrícola provocada por el predominio de explotaciones menores a 5 ha, con las características mencionadas, perdiéndose suelo con capacidad agronómica a favor del avance de la urbanización.

De esta manera se comprueba la hipótesis que guía la investigación, ya que la desaparición de explotaciones agrícolas en la zona de interfaz se explica porque los instrumentos normativos aplicados no han logrado frenar el avance urbano, al no intervenir en el mercado del suelo.

Por ser el suelo un mercado competitivo imperfecto se hace visible la relación conflictiva entre lo público y lo privado ante un Estado que no logra regular los usos del suelo. No existen criterios uniformes en la aplicación de la zonificación municipal, la forma de realizar el cálculo del avalúo fiscal debería ser revisada, se carece de instrumentos de regulación específicos para la zona de interfaz urbano-rural y de reglamentación de los espacios rurales.

Esta situación contribuye a que el inversor inmobiliario busque nuevos lugares para residencia, fuera del área urbana consolidada, de bajo precio, debido a que el valor del suelo rural es menor por la baja rentabilidad y productividad de la explotación agrícola, pero de fácil acceso a la ciudad a través de autopistas y corredores.

Frente a esta realidad, la zona de interfaz del área metropolitana de Mendoza plantea la necesidad de cambiar los objetivos políticos centrando la atención en el Ordenamiento Territorial para abordar la planificación de una manera integral en todo el territorio y no solo en el espacio urbano, utilizando instrumentos aggiornados para que efectivamente se logre frenar la expansión urbana, regular los usos del suelo y conservar suelo agrícola.

La Ley N° 8051/09 de Ordenamiento Territorial y Usos del Suelo de la provincia de Mendoza contiene este tipo de medidas a incorporar en los planes de Ordenamiento Territorial, según competencias delegadas en la Constitución provincial, como también nuevos criterios de zonificación de usos del suelo asociados a instrumentos económicos para frenar el crecimiento urbano en tierras agrícolas tales como: el ajuste del cálculo del avalúo fiscal, la determinación de la Unidad Económica Mínima (UEM) y la compensación por servicios ambientales o subsidio, instrumentos que permitirían evitar el fraccionamiento predial y preservar explotaciones agrícolas menores a 5 ha.

Si bien los resultados obtenidos no se pueden generalizar, por tratarse de un estudio de caso, permiten comprobar la aplicabilidad de la metodología a otros casos de similares características y brindan nuevos aportes a la problemática planteada. La dinámica y complejidad de una zona de interfaz urbano-rural no puede dejarse librada a las reglas del mercado de suelo. Se necesita la presencia del Estado para regular el mercado del suelo urbano y conservar suelo agrícola y para ello el Ordenamiento Territorial es la forma de planificación y gestión más adecuada para disminuir las desigualdades, evitar conflictos, mantener las condiciones de sostenibilidad urbana y rural y mejorar las condiciones de vida de quienes habitan en interfaz urbano-rural.

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Heavy metals in atmospheric dust deposited in leaves of *Acacia farnesiana* (Fabaceae) and *Prosopis laevigata* (Fabaceae)

Metales pesados en polvo atmosférico depositado en hojas de *Acacia farnesiana* (Fabaceae) y *Prosopis laevigata* (Fabaceae)

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ABSTRACT

Atmospheric dust establishes an element of study to know the distribution of pollutant particles such as heavy metals and their effects on ecological systems. The objective was to determine the elemental composition of particles deposited in two species of trees as an indicator of environmental impact in San Luis Potosí, México. The distribution of *Acacia farnesiana* and *Prosopis laevigata* trees was taken into account in five soil uses to collect leaf material and extract atmospheric dust during the spring and summer seasons, determining the concentration of heavy metals using the ICP-MS technique. The results indicated the presence of Al > Cu > Zn > Pb > V > As > Ni > Cd > Ti > Cr > Co. Correlations with values of $r^2 > 0.90$ were presented between V-Ti, Ni-V, Ni-Ti, Al-Ti and Cr-V. The species factor conditioned the concentrations of Al, Ti, V, Cr, Ni and Zn mainly in the particles deposited in *Prosopis* leaves. Particles of nine elements were conditioned by the activities of the five land uses, where the use of mineral soil affected by the presence of Al, Cd, Co, Pb, Cu and Zn. Concentrations of Cd were 6.2 times higher in the use of mining soil than in the agricultural sector; 5.9 and 5.4 times the concentrations of Co and Pb in the use of mining soil with respect to the trade and service respectively. The season had only significant effects on Cr and Pb particles. This study indicates the existence of pollutants that can affect ecological systems so it falls within the context of continued evaluation of environmental impacts.

Keywords

pollution • arboreal plants • land uses • environmental impact

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RESUMEN

El polvo atmosférico se establece como elemento de estudio para conocer la distribución de partículas contaminantes, como son los metales pesados y sus efectos sobre los sistemas ecológicos. El objetivo fue determinar la composición elemental de las partículas depositadas en dos especies de árboles como un indicador de impacto ambiental en San Luis Potosí, México. La distribución de los árboles de *Acacia farnesiana* y *Prosopis laevigata* se tomó en cuenta en cinco usos del suelo para recolectar material foliar y extraer el polvo atmosférico durante la primavera y el verano, determinando la concentración de metales pesados utilizando la técnica ICP-MS. Los resultados indicaron la presencia de Al > Cu > Zn > Pb > V > As > Ni > Cd > Ti > Cr > Co. Se presentaron correlaciones con valores de $r^2 > 0,90$ entre V-Ti, Ni-V, Ni-Ti, Al-Ti y Cr-V. El factor especie condicionó las concentraciones de Al, Ti, V, Cr, Ni y Zn principalmente en las partículas depositadas en las hojas de *Prosopis*. Las partículas de nueve elementos fueron condicionadas por las actividades de los cinco usos de la tierra, donde el uso del suelo mineral se vio afectado por la presencia de Al, Cd, Co, Pb, Cu y Zn. Las concentraciones de Cd fueron 6,2 veces más altas en el uso de suelo minero que en el sector agrícola; 5,9 y 5,4 veces las concentraciones de Co y Pb en el uso del suelo minero con respecto al comercio y al servicio, respectivamente. La temporada solo tuvo efectos significativos sobre las partículas de Cr y Pb. Este estudio indica la existencia de contaminantes que pueden afectar los sistemas ecológicos, por lo que entran en el contexto de la evaluación de los impactos ambientales.

Palabras clave

contaminación • vegetación arbórea • usos de suelo • impacto ambiental

INTRODUCTION

The atmospheric dust or particulate material constitutes an element of study to investigate the distribution of heavy metals and their effects on ecological systems and environmental health. Some authors state that they are solid or liquid particles suspended in the air, with a diverse chemical composition and a size that varies between 0.005 to 100 μm of aerodynamic diameter (27, 31). Its presence in the environment is associated with anthropogenic sources such as fossil fuels, vehicular and industrial emissions, energy production, among others (14). Studies on atmospheric dust have been limited due to the high cost of instrumental monitoring methods and also due to sampling difficulties, which has led

to the use of organisms that act as bioaccumulators (19). Several species of plants have been studied to evaluate the behavior of atmospheric dust such as: *Platanus orientalis*, *Alstonia scholaris*, *Ficus bengalensis*, *Polyalthia longifolia*, *Azadirachta indica*, *Nerium oleander*, *Lantana camara*; *Alstonia scholaris*, *Ficus bengalensis*, *Morus alba*, *Polyalthia longifolia* among others (19, 20, 26, 28).

The foliar surface of plants is continuously exposed to atmospheric patterns and is the main dust receptor affecting the reflectance spectra and growth factors of plants, so it can be used to determine the level of contamination, as well as the capacity of interception and mitigation of each species (15, 21, 24, 29).

The urban zone of San Luis Potosí - Soledad de Graciano Sánchez is characterized by the growth of the industrial population and large areas of crops under irrigation. Among the associated environmental problems are overexploitation and contamination of aquifers, erosion, salinization and loss of soil fertility and inadequate disposal of domestic and industrial waste. Emphasis is placed on the need to generate actions that address the problems related to air pollution and improve the green infrastructure (9, 15, 23).

In the urban and industrial area of the city of San Luis Potosí have been found to have levels of solid particles in suspension, with an annual average of 438 g/m³ value that is above the threshold of 90 µg/m³ marked by the WHO (World Health Organization), with the presence of elements such as Pb, Ni, Cu, Cd, As (10). Likewise, in this area, more than 20 species of trees are considered more abundant and of common use as part of the urban and regulated green infrastructure within afforestation and reforestation programs (16, 25).

In addition, several studies have been conducted that indicate the presence of heavy metals in soil, leaves and bark of tree species associated with the dynamics of agricultural, urban, industrial, commercial and service land uses.

On the other hand, the capacity of retention of atmospheric dust in species like *Shinus molle*, *Prosopis laevigata* and *Acacia farnesiana* has been studied, which has indicated differences between the seasons, species and uses of finding that the greater accumulation of atmospheric dust in leaves, occurred in the winter-spring season and the lowest in the summer, while, in the bark, the largest amount was recorded in winter and the lowest in spring (5, 6, 7, 8, 9, 13).

As a follow-up to the study of the environmental impact of land uses, the atmospheric

dust deposited in the foliar material of *Acacia farnesiana* and *Prosopis laevigata* was considered to determine the presence of heavy metals and associate it with the potential use of efficient green infrastructure in the mitigation of environmental problems such as the atmospheric contamination and the use for protection of agricultural areas.

MATERIALS AND METHODS

The study area was located in the state of San Luis Potosí, within the rural-urban area between the municipalities of "Soledad de Graciano Sánchez" and "San Luis Potosí".

The municipality of "Soledad de Graciano Sánchez", is located geographically at 22°11" North latitude and 100°56" West Longitude with altitude of 1,850 meters above sea level, while the municipality of "San Luis Potosí", at coordinates 22°09'04" North latitude and 100°58'34" West.

The climate is mainly characterized by being temperate dry with warm summer, BSkw11 (e) g. This climate registers 400 mm annual rainfall, concentrated in summer and part of autumn, particularly between the months of May to October, although it should be noted that in half of this season there is a season in which precipitation decreases.

The 6 dominant soil types are lithosol, xerosol, pheozem, chestnut tree and fluvisol (22). In a journey formed by an ecological corridor of approximately 35.5 km, it allowed locating 30 sites considering the alternation in the presence of the species *Prosopis laevigata* (Fabaceae) and *Acacia farnesiana* (Fabaceae).

The sites were located on paths fragmented by five types of dominant land use: agriculture, rural residential, commerce and services, urban and mining residential (figure 1, page 176).

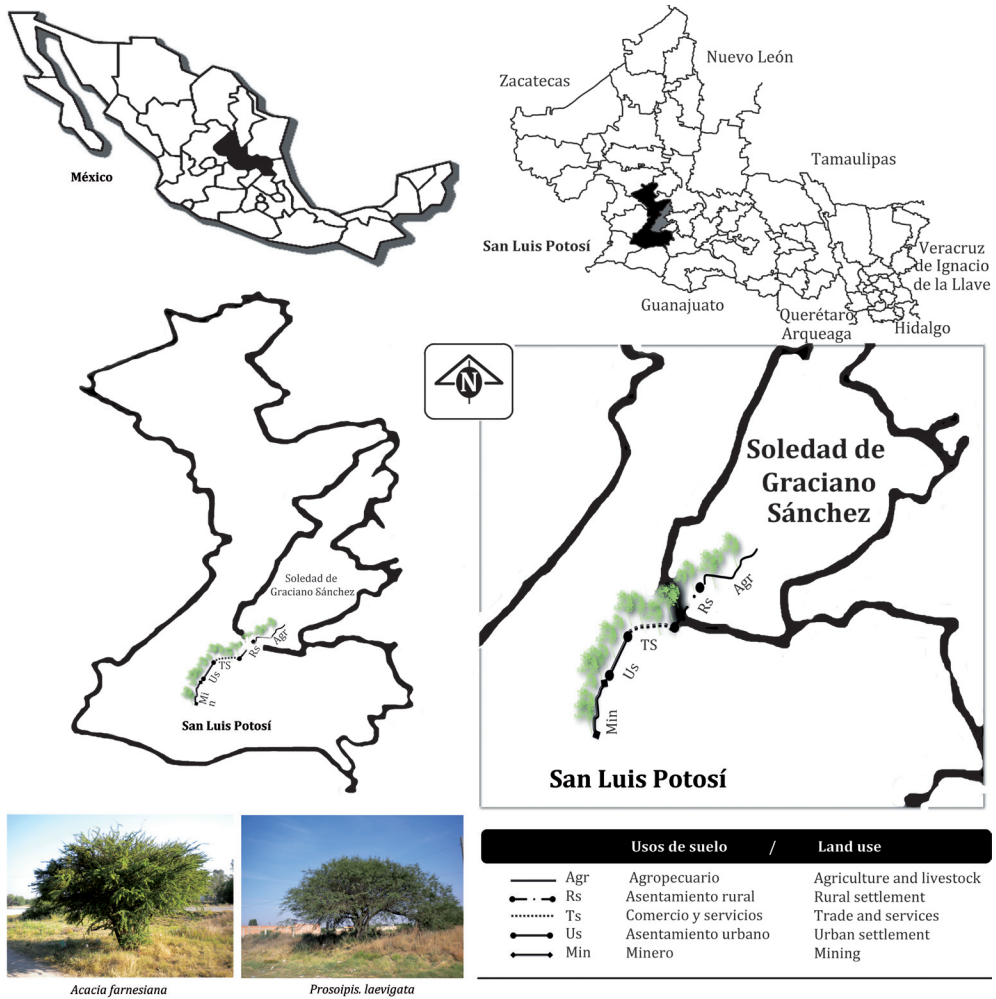


Figure 1. Location of the study area of atmospheric dust in tree species.

Figura 1. Ubicación del área de estudio de polvo atmosférico en especies arbóreas.

For each species, 30 to 40 g of foliar material were taken from branches of individuals with a height greater than 1.60 m. It was considered that the trees were located in the alignment of the road section, alternating their presence in left and right side, as well as their exposure to sources of pollution and air currents. So that there was no effect of the flowering season of these species, the samples were taken in the same individuals during the summer and autumn seasons, as well as in winter and spring, corresponding 115 samples during the four seasons. For the determination of metal concentrations, weighed between 10 to 15 g of foliar material that was washed and filtered in a 100 ml volumetric flask using a No. 42 (previously tared) Whatman filter paper, then dried in an oven at 60°C and heavy with the powder extracted from the washing of leaves.

Finally, the paper was weighed with atmospheric dust and together with filter paper used as white, in a muffle at 450°C, performing a dry digestion until

incineration. The resulting ashes were augmented to 25 ml in volumetric flask with 1% HNO₃; for the analysis of Al, As, Co, Cu, Ni, Cd, Cr, Pb, Ti, V and Zn using the ICP-MS technique. The concentrations of the metals in mg kg⁻¹ were reported. For the analysis (ANOVA), it was designed in Minitab setting an $\alpha \leq 0.05$, testing the interactions between the factors land use, species and season with respect to the concentrations of heavy metals. A correlation analysis (Pearson's correlation coefficients) was carried out, as well as a principal component analysis (PCA).

RESULTS AND DISCUSSION

The analyses carried out indicate the presence of eleven (11) metallic elements in atmospheric dust retained from a total of 115 samples of the species evaluated. The average tendency oscillated with Al > Cu > Zn > Pb > V > As > Ni > Cd > Ti > Cr > Co (figure 2).

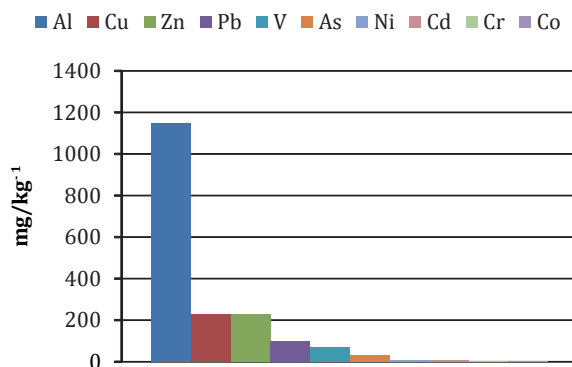


Figure 2. Average concentrations of heavy metals in sedimentable particulate matter.

Figura 2. Concentraciones promedio de metales pesados en material particulado sedimentable.

Regarding the correlation analysis, 11 significant associations were found with the presence of these elements and with values $r^2 > 0.80$ (table 1, page 179).

Among these are the relationship between V-Ti ($r^2 = 0.947$, $p = 0.000$), Ni-V ($r^2 = 0.937$, $p = 0.000$), Ni-Ti ($r^2 = 0.930$, $p = 0.000$), Al-Ti ($r^2 = 0.910$, $p = 0.000$) and Cr-V ($r^2 = 0.902$, $p = 0.000$). This observation indicates that dust contamination by metals they can come mainly from common (11).

Relationship atmospheric dust-tree species

According to the effect of the tree species factor, six of the eleven elements analysed in the sedimentary particulate material present significant differences in the concentrations of heavy metals deposited in *A. farnesiana* and *P. laevigata* (table 2, page 179). These elements were: Al ($p = 0.001$), Ti ($p = 0.000$), V ($p = 0.000$), Cr ($p = 0.000$), Ni ($p = 0.000$) and Zn ($p = 0.023$). It is distinguished that, of these six elements found in the atmospheric dust, their highest concentrations were present in the particles deposited in *Prosopis* leaves. He emphasizes that the concentrations are twice the amount of deposited particles of Ti, V, Cr, Ni and Zn in *Prosopis l.* with respect to *Acacia f.*

This result is associated with studies conducted in the area where the presence of heavy metals is determined by anthropogenic sources and the efficiency of these species to retain atmospheric dust in leaves and bark (5, 6, 7, 8).

The deposition of atmospheric dust with metallic contents implies processes such as sedimentation, impact and interception (20). Not only the diameter and shape of the particles have impacts (17).

In diverse investigations it is indicated that the dust deposition capacity of the plants can depend on its geometrical

surface, phyllotaxis and external characteristics of the leaves as it is the presence or absence of pilosity, cuticle, length of the petiole, height of the canopy and crown. Other aspects are the condition of humidity, direction and wind speed (11, 12, 18, 24). The mobilization and transport of atmospheric dust is related to variations in vegetation growth conditions, density and vegetation (2, 18, 30).

Relationship atmospheric dust-land use

The significant effect of the use of soil on the concentrations of heavy metals in atmospheric dust, was presented in nine of the 11 elements analysed, these being: Al ($p = 0.058$), Ti ($p = 0.046$), V ($p = 0.023$), Co ($p = 0.000$), Ni ($p = 0.022$), Cu ($p = 0.000$), Zn ($p = 0.000$), Cd ($p = 0.000$) and Pb ($p = 0.000$). It is distinguished that in the use of mining soil the presence of a greater quantity of particles with contents of Al, Cd, Co, Pb, Cu and Zn was evidenced (table 3, page 180).

On the other hand, particles with contents of Ni, Ti and V were concentrated in greater quantity in the use of agricultural land. It is distinguished that the concentrations of Cd were 6.2 times higher in the use of mining land than in the agricultural one; 5.95 and 5.4 times the concentrations of Co and Pb in the use of mining land with respect to trade and service, respectively. Another comparison is the one that occurred with the Zn, where they were 6.8 times their concentration in particles of the use of mining land in relation to trade and service.

The presence of these heavy metals in this area has been associated with the diversification of land uses (7, 8). Similar results regarding the effect of land uses on concentrations of heavy metals in atmospheric dust have been reported (1).

Table 1. Results of the Pearson correlation coefficient test on sedimentable particulate matter.

Tabla 1. Resultados de la prueba del coeficiente de correlación de Pearson en material particulado sedimentable.

Elemento	Al	Ti	V	Cr	Co	Ni	Cu	Zn	As	Cd
Ti	0.910									
	0.000									
V	0.887	0.947								
	0.000	0.000								
Cr	0.860	0.889	0.902							
	0.000	0.000	0.000							
Co	0.418	0.423	0.368	0.416						
	0.000	0.000	0.000	0.000						
Ni	0.847	0.930	0.937	0.878	0.436					
	0.000	0.000	0.000	0.000	0.000					
Cu	0.462	0.451	0.395	0.434	0.554	0.473				
	0.000	0.000	0.000	0.000	0.000	0.000				
Zn	0.472	0.494	0.376	0.397	0.476	0.385	0.612			
	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
As	0.260	0.245	0.193	0.257	0.269	0.281	0.700	0.404		
	0.005	0.008	0.039	0.006	0.004	0.002	0.000	0.000		
Cd	0.301	0.296	0.263	0.292	0.548	0.341	0.860	0.467	0.674	
	0.001	0.015	0.048	0.010	0.000	0.002	0.000	0.000	0.000	
Pb	0.440	0.446	0.362	0.484	0.523	0.456	0.672	0.574	0.498	0.588
	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 2. Ratio of heavy metal concentrations to deposited particulate matter according to species (N = 115, p≤0.05).

Tabla 2. Relación de las concentraciones de metales pesados en material particulado sedimentable depositado de acuerdo con la especie (N=115, p≤0,05).

Especie	Al (mg kg ⁻¹)		Ti (mg kg ⁻¹)		V (mg kg ⁻¹)	
	Media	E.E.	Media	E.E.	Media	E.E.
<i>A. farnesiana</i>	815.62	133.43	15.12	2.83	35.77	7.99
<i>P. laevigata</i>	1447.77	125.46	32.30	2.66	95.38	7.61

Especie	Cr (mg kg ⁻¹)		Ni (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
	Media	E.E.	Media	E.E.	Media	E.E.
<i>A. farnesiana</i>	2.43	0.44	4.52	0.89	255.66	92.19
<i>P. laevigata</i>	4.91	0.42	10.33	0.85	549.90	87.78

Table 3. Ratio of concentrations of heavy metals deposited in atmospheric dust according to land uses (N = 115, $p \leq 0.05$).
Tabla 3. Relación de las concentraciones de metales pesados en polvo atmosférico depositado de acuerdo con el uso de suelo (N=115, $p \leq 0.05$).

Elements	Land use											
	Agriculture and livestock		Rural settlement		Trade and service		Urban settlement		Mining			
	Average	±E.E.	Average	±E.E.	Average	±E.E.	Average	±E.E.	Average	±E.E.		
Al	1355.25	197.09	1290.12	197.09	777.52	197.09	810.12	211.53	1418.98	206.20		
Cd	2.37	1.77	2.61	1.77	2.46	1.77	4.63	1.90	14.70	1.85		
Co	1.33	0.78	1.52	0.78	0.96	0.78	1.18	0.83	5.72	0.81		
Ni	10.12	1.36	7.80	1.36	4.56	1.36	5.46	1.45	9.19	1.41		
Pb	50.80	21.87	56.38	21.87	48.92	21.87	83.92	25.93	268.52	25.28		
Cu	121.66	98.04	91.40	98.04	56.52	98.04	102.61	106.11	807.85	10.343		
Ti	29.79	4.12	26.65	4.12	14.98	4.12	18.43	4.42	28.88	4.31		
V	86.31	12.04	78.59	12.04	38.39	12.04	47.99	12.92	76.16	12.59		
Zn	217.11	138.79	236.06	138.79	156.17	138.79	331.91	148.96	1072.65	145.20		

Presence of Zn and Cu has been associated with traffic activities and industry and Ni with contaminated soils (19). Presence of this element is associated with vulcanization activities in tires, abrasion of car tires increases and lubricating oils (28).

Atmospheric dust ratio-season

With regard to the effect of the season, it is indicated that only Cr and Pb had significant effects for this factor ($p < 0.05$, figure 3). In this sense, the Cr concentrations ranged from 4.81 to 5.89 mg/kg^{-1} and in Pb from 77.54 to 125.88 mg/kg^{-1} , in both cases, in summer the highest concentration of particles of these elements.

The Cr is associated with the manufacturing industry, use or disposal of chromium products can be found in air, soil and water. This does not remain in the atmosphere, but is deposited in the soil and water (4).

As for the GDP, a large part comes from human activities such as the burning of fossil fuels, mining and manufacturing.

When the lead is released into the air, it can travel long distances before being deposited on the ground (3). Presence of Pb particles has been associated with heavy vehicular traffic and industrial dusts (1).

In scenarios where the period of dry weather, the moisture content of the soil is below the vegetation cover is poor, there is more mobilization of particles that may contain heavy metals (11, 28, 30). In the study area, it has been associated to the influence of the directions of the prevailing winds of the city vary during the course of the year in relation to particles of industrial and anthropic sources coming from the E and ENW and the influence of winds from WSW and N (10).

Principal component analysis and similarity

Respectively, the first two components (PC1 and PC2) presented a variance of 6.55 and 1.91 of the total variance, which represented 59.6% and 17.5%.

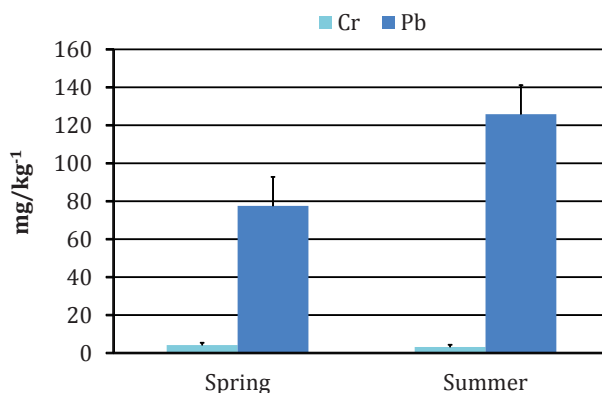


Figure 3. Relationship of concentrations of Cr and Pb in atmospheric dust according to the season.

Figura 3. Relación de concentraciones de Cr y Pb en polvo atmosférico de acuerdo con la temporada.

For PC1 the highest values of the coefficients are related to Al, Ti, V, Cr and Ni. With respect to PC2 positive coefficients were presented with respect to Co, Cu, As, Cd and Pb, and negative with Al, Ti, V, Cr, Ni and Zn. Together, the first two and the first three components represent 76.9% and 83.5%, respectively, of the total variability (figure 4). It was distinguished a group of thirteen samples distant from the axis related to the use of mining soil and with the highest averages of heavy metals found in atmospheric dust with respect to the rest, being these: 22, 24, 25, 49, 52, 53, 54, 55, 111, 112, 113, 114 and 115.

Another important group of 24 samples removed from the axis stands out at the

species level and most of them belonged to *Prosopis*. The behaviour of groups and the incidence of heavy metal localities can be attributed to anthropogenic activities derived from land uses, soil or geological composition, dust derived from vehicular traffic and streets (7, 8, 11, 26).

With respect to the similarity analysis, associations were found between the elements analysed with atmospheric dust (figure 5, page 183).

The relationship between Al, Ti, V, Ni and Cr is distinguished with values ranging from 95.08 to 96.87.

On the other hand, a group consisting of Cu, Cd, As and Pb presented values between 90.98 and 83.62 of association.

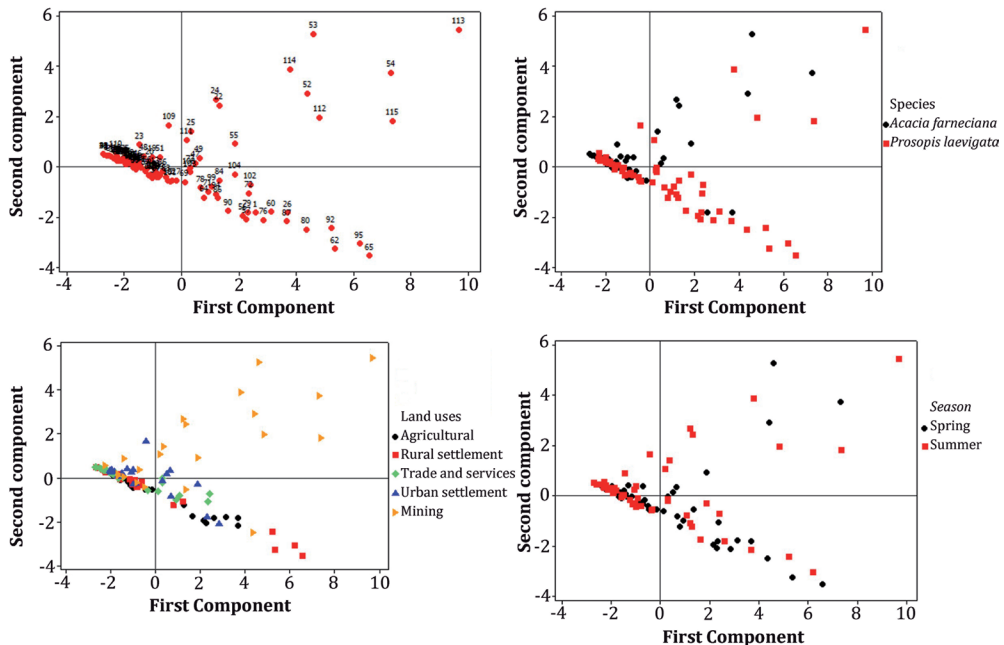


Figure 4. Dot diagram derived from the principal component analysis with atmospheric heavy metals.

Figura 4. Distribución de puntos derivado del análisis de componentes principales con metales pesados en polvo atmosférico.

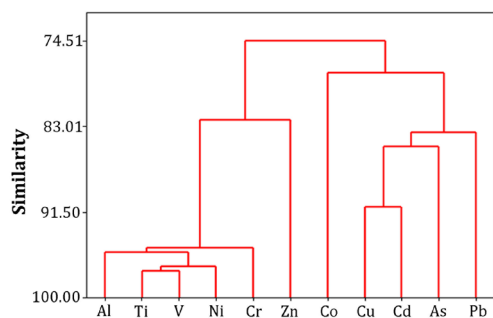


Figure 5. Dendrogram of similarity of Euclidean distance in metals studied in atmospheric dust.

Figura 5. Dendrograma de similitud de la distancia euclidiana en metales estudiados en polvo atmosférico.

This confirms a high correlation between these elements. With respect to Zn and Co, the distance found with the other elements is notorious. This indicates the association between the presence of particles and the effect of the dynamics of each of the land uses. For example, it has been associated with Pb, Cd and Cu with sources such as fossil fuel, biomass combustion and industrial emissions, vehicular traffic. Others may be associated with sources of natural contamination (18). In the study area these elements have been found in atmospheric particles and associated mainly with industrial sources such as mining activity (10).

CONCLUSIONS

The study indicated the presence of heavy metals in atmospheric dust retained in leaves of *P. laevigata* and *A. farnesiana* evidenced by concentrations that oscillated with Al> Cu> Zn> Pb> V> As> Ni> Cd> Ti> Cr> Co.

Likewise, significant correlations with values $r^2 > 0.90$ were presented between V-Ti, Ni-V, Ni-Ti, Al-Ti and Cr-V.

The species factor conditioned the concentrations of Al, Ti, V, Cr, Ni and Zn mainly in the particles deposited in *Prosopis* leaves. Particles of nine elements were conditioned by the influence of the activities of the five evaluated land uses, where the use of mineral soil affected mainly with the presence of Al, Cd, Co, Pb, Cu and Zn. Concentrations of Cd were 6.2 times higher in the use of mining land than in agriculture; 5.95 and 5.4 times the concentrations of Co and Pb in the use of mining land with respect to trade and service, respectively.

The season only had significant effects on Cr and Pb particles. This indicates the existence of contaminating elements that can affect ecological systems and environmental health considering the need to continue with these studies in the environmental impact assessment.

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Biomass function for *Acacia caven* (Mol.) Mol. distributed in the dry land areas of south central Chile

Función de biomasa para *Acacia caven* (Mol.) Mol. distribuida en áreas secas del centro sur de Chile

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ABSTRACT

Acacia caven is a key native species in the dry land region of south-central Chile. It is a species of high social and productive interest to landowners. Therefore, this study proposes a biomass function for *A. caven*, which involves incorporating predictor variables that are easily obtainable in the field and less complex than those used in already existing functions for the species. Due to the multi-purpose nature of the species and its potential for silvopastoral systems, these functions typify important planning tools to improve the management of this plant resource. To generate the biomass function, the methodology of destructive analysis of components was used on a total of 71 trees. These trees were selected, cut and weighed in the field to generate and test different models. Statistical analysis models were used and root collar diameter, diameter at breast height and total height served as predictor variables, resulting in a good adjustment of the models (R^2 adjusted the 0.97 for stem-branches, 0.90 for stems and 0.96 for total biomass), with high correlations between estimated and real values. These functions may be used safely in plant formations of *A. caven* located in the same distribution area and within the range of the used variables. In the future, however, they require validation with new measurements in other sectors than the area considered to increase geographical representativeness.

Keywords

espino • steppe • semi-arid regions of Chile • silvopastoral systems

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RESUMEN

Acacia caven, es una de las principales especies nativas presentes en el secano interior de la zona central de Chile. Es una especie que tiene un alto interés social y productivo para los propietarios de este recurso. En este sentido, el objetivo de este estudio es proponer una función de biomasa validada para *A. caven*, y que incorpore variables predictoras de fácil obtención en terreno y de menor complejidad que las consideradas en funciones ya conocidas para la especie. Dadas las características de especie multipropósito y su potencial para su uso en sistemas silvopastorales, estas funciones son importantes herramientas de planificación para mejorar la gestión y manejo de este recurso vegetacional. Para la generación de la función de biomasa, se utilizó una metodología de análisis destructivo por componentes de 71 árboles, los cuales fueron seleccionados, volteados y pesados en terreno, para posteriormente generar y probar diferentes modelos. A través de análisis estadísticos se seleccionaron modelos que utilizan como variables predictoras el diámetro a la altura del cuello, diámetro a la altura del pecho y altura total. Los resultados muestran un buen ajuste de los modelos (R^2 ajust. de 0,97 para fuste-ramas, 0,90 para ramillas y 0,96 para biomasa total), lo que indica un alto nivel de correlación entre valores estimados y valores reales. Estas funciones se pueden utilizar con seguridad en formaciones vegetacionales de *A. caven* que se ubiquen en la misma zona distribucional del recurso y dentro de los rangos de las variables utilizadas, siendo conveniente en el futuro validarlas con nuevas mediciones de sectores distintos al área considerada para estos análisis, aumentando su representatividad geográfica.

Palabras claves

espino • estepa • zona semiárida de Chile • sistemas silvopastorales

INTRODUCTION

The steppe or savanna formations, where *Acacia caven* (Mol.) Mol. ("espino") represents the dominant and co-dominant species, cover approximately 957.000 hectares in Chile (14) especially between the Coquimbo Region in the north and the Biobío Region in the south. Espino can be found in both pure formations and in association with tree species such as quillay (*Quillaja saponaria*), litre (*Lithraea caustica*), maitén (*Maitenus boaria*), huingán (*Schinus polygamus*) and others (32).

A. caven provides multiple benefits and so counts as multi-purpose species. It serves as firewood, for charcoal

production, or atmospheric nitrogen sequestration (being a leguminous species). Its ecological use in erosion control, increase of organic matter and retention of soil humidity, nutrient recycling and the creation of a favorable micro-climate for the growth of vegetation under its influence, raise its importance further (13, 20, 23).

From a productive and ecological viewpoint, *A. caven* is among the main species present in the dry land area in south-central Chile and in countries such as Argentina, Bolivia, Uruguay, Paraguay and Brazil (13).

However, mainly as a result of human pressure, it is being degraded, inducing loss of economic value of soil and vegetation (7, 13). Despite such degeneration of *A. caven* formations, their restoration has been envisaged (31) by applying silvopastoral systems as a sustainable production alternative (16, 21, 22, 24).

In line with their appearance, Espinal formations are complex with a woody stratum of varying cover and height, dominated almost exclusively by *A. caven* (25). Currently existing formations correspond to an environmental unit of mainly secondary origin (9, 13, 31); composition and structure developed as consequence of human intervention. Land preparation for agricultural use altered this resource decisively predominantly for the production of wheat and cattle, albeit later being abandoned due to market fluctuations and low productivity. Espinal cover of the resulting formations is less dense, formed by smaller individuals mainly from stump sprouts; on rare occasions, where vegetation remained free of incisive alteration, denser formations can be found (7).

In order to allow for a sustainable use of *A. caven* and considering that it mainly provides firewood and charcoal, it is necessary to count on predictor functions that make it possible to estimate the biomass this resource potentially generates. Estimations, however, remain challenging due to the high variability of the species.

Functions make it possible to estimate biomass through predictor variables which can easily be measured in the field, such as the diameter at breast height (DBH) and total height (H), or crown diameter and length, basal diameter and length of branches, producing generic (for various species) or specific (for one only) functions (3, 11, 27).

Various studies have produced biomass functions for *A. caven* in the north-central part of its natural distribution in Chile (1, 10, 26, 30). These functions, nevertheless, lose precision when applied to other regions with different conditions than those of the study area, and different ranges and parameters than the original resource (1, 6, 17, 30). Predictor variables used in these functions, and for other tree species, are mainly DBH, root collar diameter (RCD), number of commercial branches, crown diameter, trunk height, commercial height, medium crown diameter, height of maximum foliage, number of stems (1, 2, 4, 10, 26, 28, 30).

This study aims to develop a biomass function for *A. caven* present in the southern part of its geographical distribution area. The function is validated for the dry inland area of the Maule Region (Chile), considering predictor variables that are easily obtainable in the field, with less complexity than those considered in already existing functions, making them a key tool for improving the sustainable management of this native plant species.

MATERIAL AND METHOD

Location and description of the Study Area

The study area covers a surface of 2.4 hectares of adult *A. caven*, with the coordinates latitude 35°53'34" and longitude 72°08'44", within farm "San Adolfo", km 48 of "Los Conquistadores" Road, Cauquenes community, Maule Region.

Methodology for inventory and biomass measurements

The predictor function was generated using the method of direct biomass determination or destructive analysis (3). This

required a previous enumeration in the study area, during which each tree got classified as mono- or pluri-fustal (2 or more stems) according to growth habit, RCD and DBH variables and H of each shank or stem. These variables were measured on a total of 3,367 single- and multi-stem *A. caven* trees distributed across the study area.

In order to select the sample trees for the destructive analysis, the observed frequency (OF) of the resource was determined first by using the root collar diameter variable, as it represents the most common variable in native forests with characteristics like that of *A. caven* and is also recommended by Gajardo & Verdugo (10).

Furthermore, OF was determined differentially according to root collar diameter due to the existence of single- and multi-stem individuals, and to increase representativeness of the diameter classes to be sampled (figure 1 and figure 2).

After determining the OF according to growth habits, a minimum of 20 trees

was selected (35) which were distributed proportionally to diameters, with the restriction that at least one individual for each diameter class (if existing) was sampled.

In total, 71 trees (single- and multi-stem) were cut for the destructive analysis. The material extracted from these trees was classified into stem-branches -including the woody material with minimum diameter of 3 cm- and twigs-woody material with diameter below 3 cm (including leaves and fruits) (10). Both categories were weighed in the field using a metallic support device designed specifically for this purpose and scales with a maximum capacity of 200 kg ± 0.1 kg, thus obtaining the green weight of each component for each tree.

To determine the relation between dry weight and green weight and thus estimate the dry weight of the components for the generation of the biomass function, three random samples of each component were taken from five randomly selected trees, both single- and multi-stem, of the 71 cut trees.

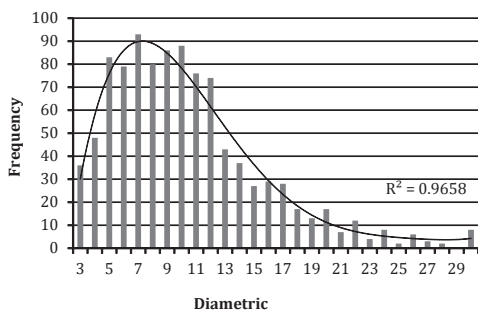


Figure 1. Observed frequency according to root collar diameter (RCD) *A. caven* single-stem.

Figura 1. Frecuencia observada según diámetro a la altura del cuello (DAC) de árboles monofustales de *A. caven*.

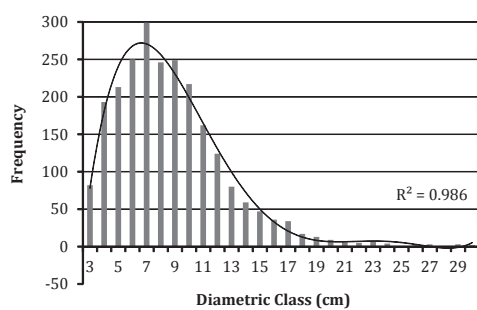


Figure 2. Observed frequency according to root collar diameter (RCD) *A. caven* multi-stem.

Figura 1. Frecuencia observada según diámetro a la altura del cuello (DAC) de árboles plurifustales de *A. caven*.

These samples were first weighed in the field (green weight) and then put into bags and labeled for dispatch to the laboratory of the Forestry Institute in Concepción.

The dry weight was obtained after drying the material in an Yih Der oven, DK-600DT model (Taiwan), with forced ventilation at $103^{\circ}\text{C} \pm 2^{\circ}\text{C}$, until obtaining steady weight between measurements every 2 or 4 hours (15). With the dry, green and total weight data the dry weight ratio (r_{sv}) per component according to growth habit (single- or multi-stem) and in total was determined, using equation 1 (3, 18). The extant literature for *A. caven* reports dry weight ratio values (r_{sv}) for stem-branches of 0.62 and for twigs 0.68 (30), so it was expected similar values for this study.

$$r_{sv} = \frac{\sum_{i=1}^n W_{s_i}}{\sum_{i=1}^n W_{v_i}} \quad (1)$$

where:

r_{sv} = dry weight ratio

w_{s_i} = dry weight of sample unit i

w_{v_i} = green weight of sample unit i

n = size of selected sample ($n \geq 1$)

The information obtained in the field and described above was then systematized and processed for generating the biomass function (BF).

On specific occasions when the stem below 1.3 m split into two or more sub-stems thus generating more than one DBH, the medium DBH (DBH_m) and equivalent DBH (DBH_e) (3) were calculated according to equations 2 and 3 respectively, and these variables were included as part of the predictor variables to determine the biomass function. Importantly, in those

trees with only one DBH (one stem) both, DBH_m and DBH_e equal DBH.

$$DBH_m = \left(\frac{\sum_{i=1}^n DBH_i}{n} \right) \quad (2)$$

where:

DBH_m = medium diameter at breast height in cm.

DBH_i = diameter at breast height of sub-stem i in cm

n = number of sub-stems

$$DBH_e = \sqrt{\sum_{i=1}^n (DBH_i)^2} \quad (3)$$

where:

DBH_e = equivalent diameter at breast height in cm

DBH_i = diameter at breast height of sub-stem i in cm

n = number of sub-stems with DBH

Adjustment and selection of biomass function (BF)

One of the objectives when selecting the models was to acquire simpler functions than the existing ones for the species (northern part of its natural distribution area). Therefore, analyzed functions contemplated only variables that are easily measurable in the field. In the process of selecting the predictor functions, DBH_e and DBH_m were considered and analyzed independently, as was assumed that one of these variables related better to the DBH variable when contemplating more than one value, because no tested model has considered these variables in conjunction. Indeed, existing models contemplate these variables only independently in relation to other variables such as H , RCD , or crown starting height and radius (upper and lower crown diameter).

When analyzing the relation between *DBH* and *H* to evaluate the coherence of the in-field data, generally the expected correlation of higher *H* with higher *DBH* was maintained. In accordance with the information from the field, the expected relation of higher diameter class related to higher biomass was confirmed. This initial analysis gave information on the reliability of the in-field data, validating it for subsequent use for determining the biomass functions.

For the generation of the dry biomass function per components and in total, simple and multiple regression models were adapted, using 12 models as a guideline (6, 11, 29, 35) for the variable type used and for each component (stem-branches, twigs & total). Moreover, 12 additional trial models were obtained through the Step-Wise method. In total 147 models were tested, considering the modification of variables, namely the use of variables as measured in the field, their squared and cubed value, transformed into natural logarithm and the combinations between them.

Selection of the final models rested on their compliance with the distributional and qualification assumptions of each model in relation to the adjusted statistic, the Mean Squared Error (*MSE*), the Prediction Error Sum of Squares (*PRESS*) and the Furnival Index (*FI*). The latter enables comparing models using transformed variables with models of non-transformed variables (19, 33, 35). Also, only models using variables with p -value < 0.05 were considered. For all analyses SPSS 21.0 software (New York, USA) was used.

RESULTS

Table 1 (page 193), shows the maximum, minimum and standard

deviation range of the state variables of the 71 trees cut (36 single-stem and 35 multi-stem) for the destructive analysis to be carried out according to the selection methodology described in section 2. In addition, the rank of the state variables of the subsample of randomly selected trees is included to calculate r_{sv} .

Table 2 (page 194), shows r_{sv} values for each component and in total, subsequently used to determine the dry weight of each of the 71 trees evaluated in the field to specify dry biomass models.

For the selection of the best models for the components among already proven models, a total of 18 models complying with the distributional assumptions were selected. These models were then qualified according to the statistics calculated for each of them in order to be able to compare them, with value 1 given to the best model according to the mentioned criteria. (For example, value 1 was assigned to the model with the best value for adjusted R^2 etc.)

Eventually, the best model selected turned out the one with the lowest total accumulated qualification (table 3, page 195).

Table 4 (page 194), shows the model selected for each component and total. Given this particular situation, the models selected were linearized applying the natural logarithm function, correction factor *Fc* was applied according to equation 4 below (2, 8, 11, 17, 36):

$$Fc = e^{MSE/2} \quad (4)$$

where:

Fc = correction factor

MSE = mean squared error

e = base natural logarithm

Table 1. Range of state variables for the trees used in the destructive analysis and calculation of R_{sv} : Minimum, maximum and standard deviation values are given according to growth habit.

Tabla 1. Rango de variables de estado de los árboles utilizados para análisis destructivo y cálculo de R_{sv} . Se muestran los valores mínimos, máximos y desviación estándar por hábito de crecimiento.

Growth habit	Evaluated trees	RCD (cm)			DBH _m (cm)			DBH _e (cm)			H (m)		
		MIN	MAX	SD	MIN	MAX	SD	MIN	MAX	SD	MIN	MAX	
		Single-stem	36	3.1	26.4	± 6.6	1.4	24.2	± 5.2	1.4	24.2	± 6.5	1.8
Multi-stem	35	3.4	24.3	± 4.9	2.2	13.2	± 3.0	2.2	18.8	± 4.1	2.0	6.2	± 0.9
Sub-sample (*)													
Single-stem	5	14.5	23.2	± 3.6	7.5	12.5	± 1.8	12.5	17.5	± 1.9	3.3	4.5	± 0.8
Multi-stem	5	10.9	19.2	± 4.1	9.1	12.3	± 1.2	10.0	17.6	± 3.2	4.0	5.8	± 0.8

(*) Sub sample of "espino" trees for the calculation and determination of dry weight ratio (R_{sv}).

(*) Sub muestra de árboles de Espino para el cálculo de la relación peso seco/peso verde (R_{sv}).

RCD = root collar diameter; DBH_m = medium diameter at breast height; DBH_e = equivalent diameter at breast height and H = total tree height.

DAC = diámetro a la altura del cuello; DAPm = diámetro medio a la altura del pecho; DAPe = diámetro equivalente a la altura del pecho y H = altura total del árbol.

Table 2. Dry weight / green weight ratio per growth habit, per component and total (value ± standard deviation).
Tabla 2. Razón peso seco / peso verde por hábito de crecimiento, por componentes y total (valor ± desviación estándar).

Type weight (Kg)	Single stem		Multi stem		Total	
	Stem-branches	Twigs	Stem-branches	Twigs	Stem-branches	Twigs
Dry weight	6.79 ± 0.46	5.16 ± 0.05	5.95 ± 0.41	5.71 ± 0.05	12.74 ± 0.43	10.87 ± 0.05
Green weight	11.46 ± 0.76	8.92 ± 0.10	10.25 ± 0.71	8.93 ± 0.04	21.70 ± 0.72	17.85 ± 0.08
r_{sv}	0.59	0.58	0.58	0.64	0.59	0.61

r_{sv} = Ratio dry weight - green weight. / r_{sv} = Razón peso seco-peso verde.

Table 4. Selection of models according to statistics.
Tabla 4. Selección de modelos de acuerdo con estadígrafos.

Selection model (*)	
Stem-branches	
Ln PFs	$= -3.6637 + 1.55606 * \ln(\mathbf{RCD}) + 0.72712 * \ln(\mathbf{H}) + 0.73537 * \ln(\mathbf{DBHe})$
Twigs	
Ln PRs	$= -2.70498 + 1.25095 * \ln(\mathbf{RCD}) + 0.89194 * \ln(\mathbf{DBHe})$
Total	
Ln PTs	$= -2.70917 + 1.50054 * \ln(\mathbf{RCD}) + 0.54516 * \ln(\mathbf{H}) + 0.70596 * \ln(\mathbf{DBHe})$

(*) Corresponding Fc has been applied to each of these models and calculated according to equation 4 (page 192).
 (*) A cada uno de estos modelos se le ha aplicado el Fc correspondiente y calculado de acuerdo con la ecuación (página 192).

Table 3. Qualification and pre-selection of models listed according to lowest total qualification conforming to four statistics
Tabla 3. Calificación de modelos preseleccionados ordenados de acuerdo con la menor calificación total según cuatro estadígrafos.

Pre-selection models	R ² Adjust		MSE		FI		PRESS		Total Rating
	Value	Rating	Value	Rating	Value	Rating	Value	Rating	
Stem-branches									
Ln PFS= -3.6637 + 1.55606*Ln(RCD) + 0.72712*Ln(H) + 0.73537*Ln(DBHe)	0.9651	1	0.0731	1	3.19	1	5.82	1	4
Ln PFS= -4.10952 + 0.44530*Ln(DBHm) + 0.94757*Ln(RCD*H)	0.9588	2	0.0865	2	3.47	2	6.35	2	8
Ln PFS= -4.03081 + 1.92693*Ln(RCD) + 0.75686*Ln(H) + 0.90835*Ln(DBHm)	0.9588	3	6.0000	7	3.47	3	6.50	3	16
Ln PFS= -3.08736 + 1.44161*Ln(RCD) + 1.08477*Ln(DBHe)	0.9558	4	0.0926	3	3.59	4	7.20	5	16
Ln PFS= -4.37264 + 2.25891*Ln(RCD) + 1.1436*Ln(H)	0.9538	5	0.0969	4	3.67	5	7.14	4	18
Ln PFS= -3.5558 + 1.84973*Ln(RCD) + 0.90835*Ln(DBHm)	0.9507	6	0.1033	5	3.80	6	7.62	6	23
Ln PFS= -3.8551 + 2.7332*Ln(RCD)	0.9217	7	0.1643	6	4.79	7	11.93	7	27
Twigs									
Ln PRs= -2.70498 + 1.25095*Ln(RCD) + 0.89194*Ln(DBHe)	0.9012	1	0.1685	1	2.95	1	12.78	1	4
Ln PRs= -3.06626 + 0.75053*Ln(RCD*H) + 0.04753*RCD	0.8965	2	0.1765	2	3.02	2	13.37	2	8
Ln PRs= -3.7002 + 1.9894*Ln(RCD) + 0.78738*Ln(H)	0.8912	3	0.1855	3	3.10	3	13.86	3	12
Ln PRs= -3.20838 + 1.90242*Ln(RCD) + 0.42735*Ln(DBHm)	0.8800	4	0.2046	4	3.25	4	15.49	4	16
Ln PRs= -3.37762 + 2.3293*Ln(RCD)	0.8735	5	0.2157	5	3.34	5	15.97	5	20
Total									
Ln PFS= -2.70917 + 1.50054*Ln(RCD) + 0.54516*Ln(H) + 0.70596*Ln(DBHe)	0.9607	1	0.0782	1	5.24	1	6.03	1	4
Ln PFS= -2.28471 + 1.41346*Ln(RCD) + 0.97263*Ln(DBHe)	0.9555	2	0.0886	2	5.57	2	6.76	2	8
Ln PFS= -3.40828 + 2.17836*Ln(RCD) + 0.95212*Ln(H)	0.9498	3	0.0998	3	5.91	3	7.38	3	12
Ln PFS= -2.75118 + 1.91584*Ln(RCD) + 0.67426*Ln(DBHm)	0.9432	4	0.1129	4	6.29	4	8.40	4	16
Ln PFS= -3.01821 + 2.58938*Ln(RCD)	0.9264	5	0.1463	5	7.16	5	10.75	5	20
Ln PFS= -1.08914 + 1.98658*Ln(DBHe)	0.9189	6	0.1613	6	7.52	6	12.22	6	24

RCD = root collar diameter (cm); DBH_m = medium diameter at breast height (cm); DBH_e = equivalent diameter at breast height (cm); H = total tree height (m); PFS = dry weight of stem-branches (kg); PRs = dry weight of twigs (kg); PFS = total dry weight (kg); R² Adjust= adjusted determination coefficient; MSE = mean squared error; FI = Furnival Index; PRESS = Prediction Error Sum of Squares. Value p<0.05.

DAC = diámetro a la altura del cuello (cm); DBH_m = diámetro a la altura del pecho medio (cm); DAP_e = diámetro a la altura del pecho equivalente (cm); H = altura total del árbol (m); PFS = peso seco del fuste-ramas (kg); PRs = peso seco de las ramillas (kg); PFS = peso seco total (kg); R² Adjust= coeficiente de determinación ajustado; CME = cuadrado medio del error; IF = Índice de Furnival; PRESS = suma de los cuadrados de los predichos. Valor p<0.05.

Subsequently, these models were submitted to a comparative analysis charting the dry weight values of observed (real) biomass in relation to the dry weight of estimated biomass (figure 3), to determine whether the selected models under- or overestimate tree biomass (8, 35). If the models measure adequately, the charted dots should run along or near line $y = x$.

DISCUSSION

The enumeration carried out at the beginning helped proving the high variability of this species. Although in general the expected growth tendencies were confirmed, *e.g.*, a higher RCD equals

more biomass, this did not always hold, because mechanical damage (broken branches, rot, signs of decay, etc.) of some individuals affected their normal production and consequent biomass.

The use of the observed frequency criterion according to the RCD variable, following Gajardo & Verdugo (1979), for the selection of trees to be cut guaranteed high reliability of the model selection during the sampling process.

Regarding the easy measurement of the variables used in the models, it should be noted that *e.g.*, Gonzalez-Benecke *et al.* (2014) indicate that in order to obtain estimations that are more precise and representative models, other variables, like age, wood density and/or productivity, should be included.

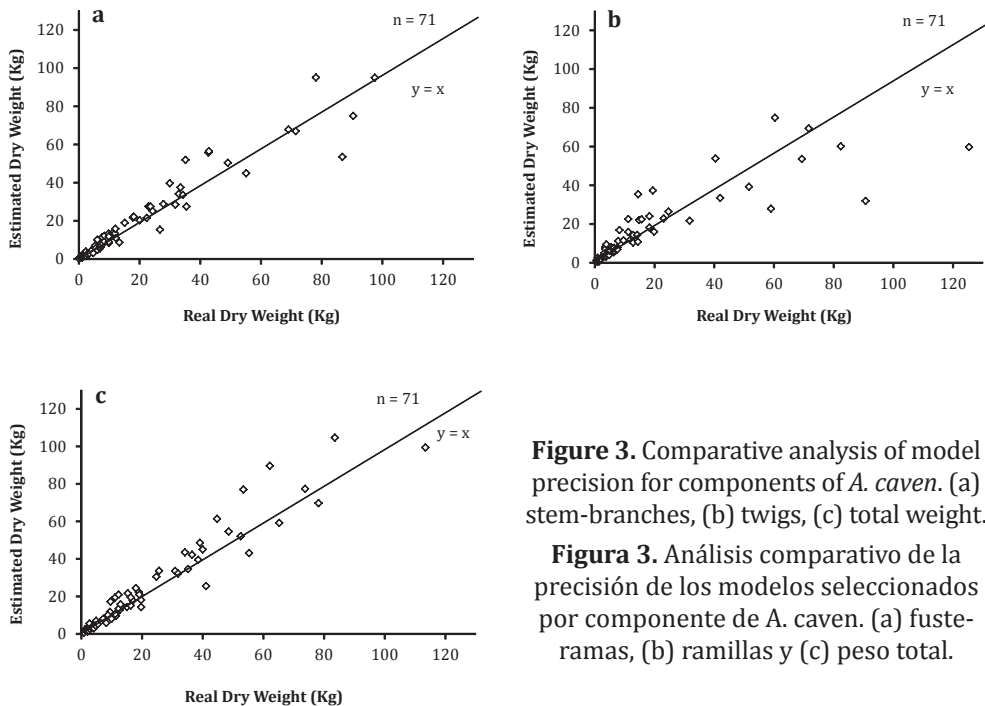


Figure 3. Comparative analysis of model precision for components of *A. caven*. (a) stem-branches, (b) twigs, (c) total weight.

Figura 3. Análisis comparativo de la precisión de los modelos seleccionados por componente de *A. caven*. (a) fuste-ramas, (b) ramillas y (c) peso total.

However, these variables complicate the actual applicability of the models in the field, and make in-field measurements difficult, considering in particular the great diversity of the species. Thus it was preferred sticking to variables which can be applied easily.

Combining the information obtained in the field with database analysis facilitated the generation of more than 147 preliminary models, based on the most commonly reported models found in the literature (6, 11, 29, 35). It also reinforced this confidence in the methodology proposed by Segura & Andrade (2008) utilizing the models selected for each component, and determine which one best adapts to the production of aerial biomass of *A. caven* in this particular region.

Out of these models, 18 with values of adjusted R^2 between 87% and 97% ($p < 0.05$) were pre-selected, resulting in good to very good data adjustment (table 3, page 195). Also, the selected functions consider the use of easy-to-use regression variables such as RCD, DBH_e and H which can be obtained effortlessly in the field (table 4, page 194) (8). They also strengthen the models' reliability and usability, making it superior to other models, which consider variables such as RCD, medium DBH of sprouts, height of maximum foliage, upper and lower crown diameter and total height (13).

The proposed models (table 4, page 194) do not differentiate between single- or multi-stem individuals, although initially the sampling methodology had considered this differentiation.

The models obtained consider each sapling as an individual, no matter if several are part of one stump (multi-stem). This kind of model makes it necessary to measure the total height of each existing sapling, which may imply more measuring

work, but also leads to higher representativeness than the in-field estimation of medium height of multi-stem individuals.

Moreover, with the existing technology of dasometric instruments, and as conducted in this study, the variable can be measured quickly, allowing the use of H as estimator for the two growth habit categories.

Also in table 4 (page 194), the lower values of adjusted R^2 correspond to the models related to the twigs component with values between 0.87 and 0.90, which was expected considering the difficulty of finding a model for the crown biomass with state variables such as RCD and DBH_e , and the high variability of the resource in the field.

The higher adjusted R^2 values are found in the stem-branches and total components, which indicates that biomass estimations of these components relate better to the state variables of the trees, confirming Segura & Andrade's (2008) finding in relation to biomass model determination. They point out that a high correlation of the models for stem and branches with variables such as DBH is expected, but not so with leaves and twigs.

Regarding precision of the models it turns out that the models for stem-branches and total components are more precise (figure 3a and figure 3c, page 196), although some over- and under estimations occur as biomass production increases. This has also been discussed by Segura & Andrade (2008).

In the case of the twigs component (figure 3b, page 196), the model is acceptable for lower values, but tends to underestimate biomass for higher values. It was believed that this tendency may prove significant in relation to the obtained frequency for individuals with RCD longer than 20 cm (on average one individual per diameter class), although this almost never occurs.

This makes it more difficult to elaborate a model for these diameter classes compared to individuals with a lower RCD, which were well represented in the in-field sampling process, again confirmed via the three models' perfectly acceptable precision level, both for lower and medium biomass production. Additionally, the studentized residue dispersion graphs (figure 4) show that the dot clouds have a random behaviour around 0, which indicates that the data fit properly to the models.

It should be noted that the selected models by design do not comply with additivity property, although for Dieguez *et al.* (2003) this counts as one of the most important properties models should comply with. Yet, since the selected models are log-linearized, Cancino's (2006) argument holds, stating that the summed-up biomass of the estimated

components for this type of model does not equal total estimated biomass, so they cannot be harmonized, and the additivity concept is not admissible in these particular situations.

In relation to the better adjusted variables (RCD, DBH_e & H), it was rightly expected that RCD adjusts well, as this variable has been representative for characterizing these plant formations (10). DBH_e variable adjusted better than DBH_m in all selected models, pointing to higher representativeness than real DBH, as it produces better ponderation of the different DBH measured in the field than their simple measurement (Eq. 1 and 2, page 191). In the case of H it was also to be expected to rely on this variable in the stem-branches and total models, as this variable normally associates directly to biomass production-higher H equaling more biomass production.

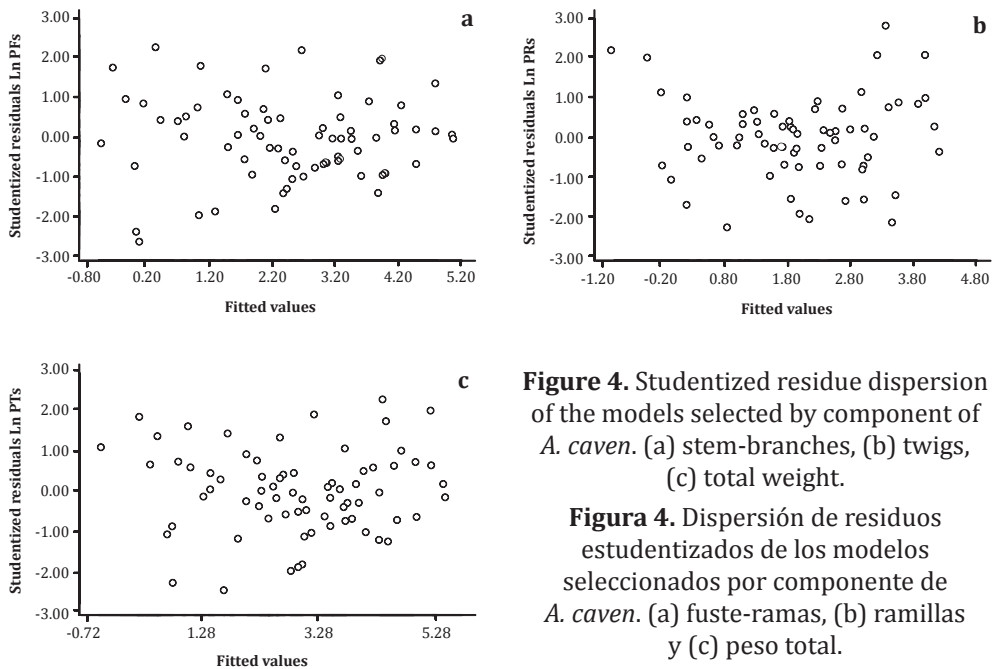


Figure 4. Studentized residue dispersion of the models selected by component of *A. caven.* (a) stem-branches, (b) twigs, (c) total weight.

Figura 4. Dispersión de residuos estudentizados de los modelos seleccionados por componente de *A. caven.* (a) fuste-ramas, (b) ramillas y (c) peso total.

A literature review on the determination of biomass in *Peumus boldus* (6, 11, 12) reveals that the state variables that best explain biomass production of the different species analyzed here can be multiple, but they also affirm that easily measurable variables such as *RCD*, *DBH* and *H* are more characteristic and representative.

In the selected models the coefficient of determination adjusted R^2 is high, showing values of 0.97 for stem-branches, 0.90 for twigs and leaves, and 0.96 for total biomass, indicating a high level of correlation between estimated and real values. These values are similar to the R^2 values presented by Aguirre & Infante (1988), Prado *et al.* (1988) and INFOR (2012) for the stem-branches (between 0.93 and 0.97), twigs (0.92) and total (between 0.96 and 0.98) components.

CONCLUSIONS

The initial characterization of the resource (enumeration) produced good information regarding distribution according to diameter classes, which substantially improved the sampling of the trees to be cut and their representativeness in what followed. Although methodology of biomass analysis with the destructive sampling process involves high costs, it still serves well to obtain this

type of functions, which becomes clear considering the good adjustment level obtained in the selected models.

These functions, with the used methodology and adjustments, can reliably be used in formations of *A. caven* in the distribution area of the dry inland region between the Cauquenes (VII Region) and Chillán (VIII Region) communities within the range of the state variables used in this study (table 1, page 193). However, it is recommended to validate and adjust them with new measurements of trees from other regions, too, to get a higher level of geographical representativeness and reliability.

These new biomass functions with a high correlation level are a new tool for the forestry and agricultural sectors helping to better plan and manage this important resource in the dry land regions of south-central Chile.

The potential of *A. caven* formations for the application of silvopastoral systems is well known, partly because it is a multi-purpose species that facilitates interactions between trees, grassland and livestock. In this context the new functions generated and proposed in this study allow for a more adequate and reliable assessment of the tree component before commencing silvopastoral management, and may also support new research to optimize these systems and the interrelations between their components.

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Nutritional parameters of "mate cocido" with milk prepared from two species of *Ilex* and their commercial mixture for school age population

Parámetros nutricionales del "mate cocido" con leche preparado a partir de dos especies de *Ilex* y su mezcla comercial, para la población en edad escolar

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ABSTRACT

The infusion at 1.5% of yerba mate (*Ilex paraguariensis* A.St.-Hil.) (Ip) corresponds to "cooked mate" (MC), whose combination with milk (50:50 v/v) (M) and refined sugar (S) is the "mate cocido with milk", a popular food in southern South America offered to preschool and primary education students (3-13 years old) in Argentina. Another species of the genus, *I. dumosa* Reissek (Id), has low caffeine; appears in the market as an Id:Ip (70:30 w/w) mixture. The substitution of Ip by the mentioned mixture maintains analogous nutritional values and significantly lowers the caffeine levels ($\alpha=0.05$); this allows for a higher consumption of the food during the day, without reaching the xanthine daily limits (40-100 mg day⁻¹) for the age range, favoring the students intellectual and physical performance without adverse effects. The daily intake of a double serving (breakfast and afternoon snack) of MC+M+S provides high energy and is a source of carbohydrates, proteins, vitamins A, B₁, B₂, B₅, B₆, and B₁₂, biotin and essential minerals (Mn, P, Zn, Cu, Ca, Mg, Na, Cl, K, Fe and Al), with low cholesterol. The nutritional parameters are within international limits. Heavy metals do not make it to the infusion or they remain well below harmful values.

Keywords

"Chá mate" • *Ilex dumosa* • *Ilex paraguariensis* • "Mate cocido" • "Mate cocido" with milk • nutritional value • schoolchildren diet

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RESUMEN

La infusión al 1,5% de "yerba mate" (*Ilex paraguariensis* A.St. -Hil.) (Ip) constituye el "mate cocido" (MC), que con leche (50:50 v/v) (M) y azúcar refinada (S) es el "mate cocido con leche", alimento popular en el Cono Sur de América ofrecido en la escuela a niños de educación inicial y primaria (3-13 años) en Argentina. Otra especie del género, *I. dumosa* Reissek (Id), tiene baja cafeína; se presenta en el mercado en mezcla con Ip (Id:Ip 70:30 p/p). Sustituyendo Ip por la mezcla, el MC+M+S mantiene valores nutricionales análogos con niveles significativamente bajos de cafeína ($\alpha=0.05$); aún con consumos diarios altos de este alimento, no se alcanza los límites de xantinas establecidos para el rango etario (40-100 mg día⁻¹), favoreciendo el desempeño intelectual y físico sin efectos adversos. La ingesta diaria de una doble porción (desayuno y merienda) de MC+M+S aporta altos valores energéticos y es fuente de carbohidratos, proteínas, vitaminas A, B₁, B₂, B₅, B₆ y B₁₂, biotina y minerales esenciales (Mn, P, Zn, Cu, Ca, Mg, Na, Cl, K, Fe y Al), con bajo colesterol. Los parámetros nutricionales están comprendidos dentro de límites internacionales. Los metales pesados no pasan a la infusión, o están muy por debajo de los valores peligrosos.

Palabras clave

"chá mate" • *Ilex dumosa* • *Ilex paraguariensis* • "mate cocido" • "mate cocido" con leche • valor nutricional • dieta escolar

INTRODUCTION

"Yerba mate" or "erva mate" (hereinafter, YM) is a plant food product made from shoots and leaves of *Ilex paraguariensis* A.St.-Hil. (Fam. Aquifoliaceae) once they are toasted, dried, milled and conditioned (6, 26). It is wanted for its nutritional value, highlighting its content in xanthines, some vitamins and minerals, and chlorogenic acids. Its infusion is the most traditional non-alcoholic beverage in the southern Cone of Latin America (Argentina, southern Brazil, Paraguay and Uruguay, and partially Chile), and is regulated by its respective Food Codes and specific provisions (3, 6, 7, 26, 37, 38, 39, 48).

The first three countries are the only producers of YM in the world, and half of this production takes place in Argentina, on a surface of 165,000 ha, with an annual yield of 820,000 t of "green leaves",

from which 37,000 t of produced YM are exported annually (23).

The most frequent forms of YM consumption are brewed mate (in a hollowed pumpkin or another container), "tereré" (a cold maceration), and the tea-like infusion known as "mate cocido" (in Spanish) or "chá mate" (in Portuguese), which has recently gained wide dissemination among the rural and urban population in the region.

"Mate cocido" (hereinafter, MC) is obtained by adding approximately 1 L of recently boiled water to 20-30 g of YM; the resulting infusion is then filtered and usually sweetened with cane sugar or honey. "MC in a cup" has recently gained wide acceptance due to its easy preparation using a porous paper "sack" or "bag" (39) containing approximately 3 g of

YM, over which around 200 mL of recently boiled water are poured, and then usually sweetened. In both cases, MC with milk is prepared by adding cow milk in variable proportions, significantly increasing the infusion nutritional value. This product is a food supplement offered more frequently in schools to students between 3 and 13 years old, in the preschool and primary education sector in Argentina. With its administration, it is intended to mitigate factors that determine nutritional deficiencies and low-quality diet of children in Argentina, as response to the food insecurity of disadvantaged sectors of the population (4, 5).

A related species, *Ilex dumosa* Reissek, commonly known as "dumosa", "caá mirí" or "yerba señorita", has been included in the Argentine Food Code in the group of "herbs for infusions" (CAA 2017: Cap. XV, Art. 1192). It is of interest due to its low xanthine content, so that its cultivation has been promoted in the recent years (42, 43). It has been demonstrated that a mixture in a 70:30 w/w proportion of *I. dumosa* and *I. paraguariensis* shows a great part of the organoleptic characteristics of YM made from *I. paraguariensis*, with a slightly lower caffeine proportion due to the predominance of *I. dumosa*; currently, it is a commercial product that satisfies a wide range of consumers (29).

The goal of the present study is to assess the comparative nutritional value of MC with milk obtained from the *Ilex* species mentioned above, proposing as alternative the use of the 70:30 w/w mixture of *I. dumosa*:*I. paraguariensis* to obtain a low-caffeine food product with good nutritional quality.

MATERIALS AND METHODS

Materials

The following samples, from the Argentine market, were used: a) commercial YM "sacks" from 6 different brands (3 from the province of Misiones and 3 from the Province of Corrientes), acquired in shops from San Luis and Corrientes, with around 3 g each, made from *I. paraguariensis* A. St.-Hil. (hereinafter, Ip); other similar samples were prepared in the laboratory from *I. dumosa* Reissek (hereinafter, Id), and separately from a commercial 70:30 w/w mixture of *I. dumosa* + *I. paraguariensis* (hereinafter, Id+Ip mix), whose crude drug was provided by the Establishment "Las Marías" (Gobernador Virasoro, Province of Corrientes); b) commercial samples of whole cow milk, sterilized by ultra-high temperature, from the dairy basin of the Argentine center-east (hereinafter, M); and c) commercial refined sugar (hereinafter, S) from the province of Jujuy.

Samples mentioned in b) and c) were acquired in the local market of San Luis. All samples were included within the framework of the Argentine Food Code requirements (6), and the reactants used were of analytical grade.

Extracts

The aqueous extracts were prepared according to the procedure described in Maiocchi *et al.* (2016), which represents the common conditions of use and the manufactures recommendations, by pouring 200 mL of recently boiled deionized water (ca. 84° C) over a sack of 3 g YM of each species and their mixture, leaving them in contact with the water for 5 min. The resulting infusions, at 1.5%, were filtered through Whatman n° 5 filter paper. The MC with milk was prepared by

mixing the 1.5% infusions of Id, Ip and Id+Ip mix with whole milk (M) in a 50:50 (v/v) proportion, and adding 10 g of refined sugar (S), equivalent to two teaspoons.

Analytical methods

The 1.5% infusions (MC) and the MC+M+S mixtures were mostly analyzed using the A.O.A.C. methods (21) for hygroscopic moisture, lipids, ashes, crude protein and chlorogenic acids; total dietary fiber was evaluated by the N^o 20511 IRAM Norm (25); and total nitrogen was assessed by the Kjeldahl method.

Total carbohydrates (non-nitrogenous extractive substances) (17), in grams per serving, were calculated by the difference with respect to the real extract. Calories per portion (in kcal and kJ) were calculated using the Atwater conversion factors (33). Caffeine was determined by UV spectroscopy (method of reference in Argentina) (27) as well as by HPLC (24).

Fat-soluble vitamins (A, D, E, K and provitamin A) and some water-soluble vitamins of the B complex (B₁, B₂) were determined by high performance liquid chromatography with a HPLC-UV/DAD UltiMate[®] 3.000 chromatograph (Dionex), Gemini C18 column (Phenomenex[®]), mobile phase acetonitrile:phosphoric acid 0.05% (20:80), flow of 0.8 mL min⁻¹, at a 330 nm wavelength; the UV spectra were collected within the 200-367 nm interval, comparing with Sigma[®] standard patterns and processing the data with the software associated to the instrument (Chromleon[®]); while vitamins B₅, B₆ and B₁₂, biotin and niacin were evaluated by microbiological methods (44).

The elemental composition of the infusions and mixture was established by the samples mineralization as described by Abou-Arab & Abou-Donia (2000), with some modifications (31); the concentration of 32 selected chemical elements and an internal standard (Indium) was

determined by ICP-OES (inductively coupled radial spectrometer, Varian[®]-Pro Ser. EL-05083717), with a Czerny-Turner[®] monochromator, a holographic diffraction grid and a VistaChip CCD (charge coupled device) array detector, calibrated quarterly, and using a Kretz[®] 5530 precision calibrated scale. Shown in table 1 (page 207), are the wavelengths used and the limits of detection for each analyzed element.

Serving size and daily average intake

The serving size consisted in a medium cup of 200 mL capacity (30), both for the 1.5% infusion (MC) as well as for the MC+M+S combination. The daily intake was set at 2 servings per person per day, that is, 400 mL of MC with milk and sugar, corresponding to breakfast and afternoon snack, just like it is commonly provided to children at home and/or schools.

Nutritional value

It was calculated based on the requirements of children between 3 and 13 years old, according to the most accepted international parameters, such as the Dietary Reference Intakes (DRIs), Recommended Dietary Allowance (RDA), Adequate Intake (AI) and Tolerable Upper Intake Level (UL) (6, 11, 15, 16), in addition to the Estimated Dietary Intakes (8) and the Typical Daily Nutritional Intake (36), taking into account the levels of nonobservance of adverse effects (NOEA in Spanish), that is, "the maximum intake of a determined nutrient not associated with adverse effects" (6).

Statistical analysis

Experimental design had 5 repetitions. The data of samples and formulas were analyzed using the *Infostat* program (10), by means of one-way ANOVA, followed by a comparison of averages (LSD test, $\alpha=0.05$); in all cases the p-value was lower than 0.0001.

Table 1. Wavelength (λ) and limit of detection for each element.

Tabla 1. Longitud de onda (λ) y límite de detección para cada elemento.

Element	Ag	Al	As	B	Ba	Ca	Cd	Co
Wavelength (nm)	328.068	308.215	193.696	249.773	493.408	317.933	226.502	228.615
Detection limit (mg kg ⁻¹)	0.021	0.06	0.105	0.009	0.003	0.021	0.003	0.006

Element	Cr	Cu	Fe	Hg	K	Li	Mg	Mn
Wavelength (nm)	267.716	324.754	259.940	194.164	766.491	670.783	279.800	257.610
Detection limit (mg kg ⁻¹)	0.012	0.009	0.012	0.03	0.45	0.006	0.06	0.003

Element	Mo	Na	Ni	P	Pb	Sb	Se	Si
Wavelength (nm)	202.032	588.995	231.604	213.618	220.353	206.834	196.026	251.611
Detection limit (mg kg ⁻¹)	0.015	0.06	0.03	0.15	0.084	0.063	0.15	0.06

Element	Sn	Sr	Th	Ti	Tl	U	V	Zn
Wavelength (nm)	189.927	421.552	401.913	334.947	377.572	409.013	292.401	213.857
Detection limit (mg kg ⁻¹)	0.051	0.001	0.09	0.006	0.081	0.45	0.009	0.006

RESULTS AND DISCUSSION

Nutritional parameters

The nutritional contributions to the daily reference intake (DRI) of one serving of "mate cocido" with milk and sugar, made from both species of *Ilex* and their mixture, are comparatively shown in table 2 (page 208-209).

None of the three formulations present significant differences for most of the analyzed nutritional parameters, although they do differ significantly in the concentration of caffeine and chlorogenic acids (table 2, page 208-209). In the Id: Ip mixture case, the nutritional values are directly related with the proportion in which both species appear in the commercial product.

Taking into account the energy needs of the age range between 3 and 13 years old (the considered schoolchildren age), between 1050 and 2550 kcal have been established (14); the daily intake of two servings of the studied products provides 8-22% of the required calories, while it also supplies up to 58% of the required

proteins and 22-26% of carbohydrates. Regarding vitamins, such intake doubles or triples the daily requirements (12) of biotin, provides 44-96% of cyanocobalamin, 30-76% of riboflavin, 10-38% of thiamine, 20-32% of pantothenic acid, 14-28% of vitamin A and 9-26% of pyridoxine. Due to the 50:50 v/v dilution, the total fat is half of the supplied by an equal intake of whole milk, with a proportional decrease in the cholesterol value. All these amounts are below the NOEA (levels of nonobservance of adverse effects) limits established as safe, regarding vitamins, by the Argentine Food Code (6).

The same occurs with the other nutritional factors, when compared with the allowed maximum intake levels (15). Finally, it is worth mentioning that these formulas do not provide dietary fiber.

Even though caffeine is not required as a nutritional principle, it is considered here due to the stimulating effect generally caused by xanthines on the CNS.

Table 2. Nutritional value per serving (200 mL) of "mate cocido" with milk and sugar, obtained from *Ilex paraguariensis* (Ip), *I. dumosa* (Id) and the Id:Ip (70:30 w/w) mix.
Tabla 2. Valor nutricional por porción (200 ml) de "mate cocido" con leche y azúcar, obtenido de *Ilex paraguariensis* (Ip), *I. dumosa* (Id) y la mezcla Id:Ip (70:30 p/p).

Nutritional parameters	Units	DRI	MC _{Ip} +M+S		MC _{Id} +M+S		MC _{mix} +M+S	
			200 mL ^(a)	%DV	200 mL ^(a)	%DV	200 mL ^(a)	%DV
Energetic value	kcal	1050-2550	104.2±8.79	4-11	103.9±9.69	4-11	104.0±7.64	4-11
	kJ	4390-10660	436.2±36.8		435.2±40.6		435.5±31.9	
Water	mL	1700-2400	189.8±27.6	7-13	189.7±32.25	7-13	189.7±29.8	7-13
Proteins	g	15.5-44	3.28±1.18	5-29	3.27±0.235	7-23	3.28±0.16	7-22
Total fat	g	Und.	3.37±1.21	und.	3.37±1.21	und.	3.37±1.21	und.
Cholesterol	mg	the lowest possible	10.31±5.86	und.	10.31±6.24	und.	10.31±0.60	und.
Carbohydrates	g	130	15.48±0.9	11-13	15.41±1.41	11-13	15.43±1.12	11-13
Total dietary fiber	g	19-26	0	0	0	0	0	0
Vitamin A	µg	400-600	50.0±5.6	7-14	49.6±3.7	8-13	49.7±4.77	7.5-14
Thiamine (B ₁)	mg	0.6-0.9	0.072±0.03	5-17	0.071±0.042	3-19	0.071±0.03	4.5-17
Riboflavin (B ₂)	mg	0.6-0.9	0.185±0.04	16-37	0.182±0.045	15-38	0.183±0.034	16-36
Niacin (B ₃)	mg	8-12	0.128±0.02	0.9-1.8	0.123±0.015	0.9-1.7	0.125±0.03	0.8-2
Pantothenic acid (B ₅)	µg	3-4	0.447±0.035	10-16	0.450±0.02	11-16	0.449±0.025	11-16
Pyridoxine (B ₆)	mg	0.6-1	0.060±0.015	4.5-12	0.057±0.01	5-12	0.058±0.02	4-13

Folic acid (B₉)	µg	200-300	6.34±0.88	2-4	6.36±1.065	2-4	6.35±0.915	2-4
Cyanocobalamin (B₁₂)	µg	1.2-1.8	0.480±0.06	23-45	0.483±0.09	22-48	0.482±0.07	23-46
Vitamin C	mg	25-45	1.081±0.18	2-5	1.083±0.209	2-5	1.082±0.195	2-5
α-tocopherol (vit. E)	mg	7-11	0.075±0.02	0.5-1	0.075±0.02	0.5-1	0.075±0.02	0.5-1
Vitamin K	µg	55-60	0.316±0.04	0.5-0.6	0.316±0.04	0.5-0.6	0.316±0.04	0.5-0.6
Vitamin D	µg	15	0.038±0.01	0.2-0.3	0.038±0.01	0.2-0.3	0.038±0.009	0.2-0.3
Biotin	µg	12-20	20.0±3.20	84-193	20.0±3.2	84-193	20.0±3.2	84-193
Carotenoids	µg	und.	18.5±1.6	und.	18.5±1.6	und.	18.5±1.6	und.
Other physico-chemical parameters								
Dry extract	g	-	22.87±3.16	-	22.83±2.73	-	22.84±3.55	-
Total ashes	g	-	0.805±0.18	-	0.793±0.148	-	0.796±0.139	-
Total nitrogen	g	-	0.574±0.149	-	0.557±0.176	-	0.559±0.166	-
Caffeine	mg	45-85 ^(b)	22.4±1.85 (A)	-	1.94±0.16 (B)	-	8.04±0.26(C)	-
Chlorogenic acids	g	-	0.14±0.006(A)	-	0.06±0.003(B)	-	0.09±0.004(C)	-

MC_{ip}: "mate cocido" from *Ilex paraguariensis* (Ip); **MC_{id}**: "mate cocido" from *I. dumosa* (Id); **MC_{mix}**: "mate cocido" from 70:30 (w/w) mix of Id+Ip; **M**: sterilized whole milk (UHT); **S**: refined sugar; **DRI**: daily reference intake (covers nutritional requirements of entire population between 3-13 y/o, based on DRIs (Dietary Reference Intakes, www.nap.edu) and others (6, 8, 36)). ^(a) **servings size**: a medium cup (100mL MC+100mL M+10g S = ca. 200mL). ^(b) according to Nawrot et al. (2003) and Health Canada (2012). **%DV**: range of the Dietary Value covered by a serving of "mate cocido" +M+S for a daily diet of 1050-2550 kcal or 4390-10660 kJ (14). **und.**: undetermined. **(A) (B) (C)**: the means with different letters indicate statistically significant differences (for each parameter) at 0.05 level.

MC_{ip}: "mate cocido" de *Ilex paraguariensis* (Ip); **MC_{id}**: "mate cocido" de *I. dumosa* (Id); **MC_{mix}**: "mate cocido" de la mezcla 70:30 (p/p) de Id+Ip; **M**: leche entera esterilizada (UAT); **S**: azúcar refinado; **DRI**: ingesta diaria de referencia (cubre requerimientos nutricionales de la población entre 3 y 13 años, basada en las DRIs (Dietary Reference Intakes, www.nap.edu) y otras (6, 8, 36)). ^(a) **tamaño de porción**: una taza mediana (100ml MC+100ml M+10g S = ca. 200ml). ^(b) según Nawrot et al. (2003) y Health Canada (2012). **%DV**: % del Valor Dietario cubierto por una porción de "mate cocido" +M+S para una dieta diaria de 1050-2550 kcal ó 4390-10660 kJ (14). **und.**: indeterminado. **(A) (B) (C)**: las medias con diferentes letras indican diferencias estadísticas significativas (para cada parámetro) a un nivel de 0,05.

These bioactive principles contribute, in certain extent, to a better school performance, both intellectual as well as physical (2, 19, 22, 28, 45, 47), in particular when they are part of the breakfast provided in schools, since in many cases it is the first food intake of the day (40, 41), and present a strongly dose-dependent pharmacokinetics and psychomotor effects.

However, children compose a group of risk in which high doses of caffeine can provoke adverse effects (45, 46). Although there are no established international limits, it is considered that the daily consumption of caffeine in children and teenagers should not exceed 2.5 mg kg^{-1} of body weight, whereas for adults, it should be below 400 mg day^{-1} (18, 20, 35).

Assuming that only YM provides caffeine to the MC+M+S, the one made from Ip reached 20-25 mg of caffeine per serving. This value is similar to that from diverse sources regarding tea infusions (c. $23 \pm 12 \text{ mg}$), and 2-3 times lower than the indicated for the coffee infusion (c. $57 \pm 20 \text{ mg}$) under the common preparation conditions (20, 22, 32, 34). Regarding the MC+M+S made from Id (with 1.5-2.5 mg of caffeine/100 mL), it had, on average, 11 times less caffeine than Ip, while the Id:Ip (70:30 w/w) commercial mixture (with 7-9 mg/100 mL) had, in average, 2.75 times less caffeine than YM with Ip.

Table 3 and figure 1 (page 211), show the limits of caffeine consumption and of potential servings of MC+M+S per day for the considered age groups, in comparison to those of tea with milk and coffee with milk.

A diet including 2 medium cups (of 200 mL) per day of MC made with *Ilex paraguariensis* with milk (50:50 v/v) and sugar, involves a caffeine intake of c.

$40\text{-}48 \text{ mg day}^{-1}$, similar to the provided by the same amount of tea with milk, and well beyond the limits established for the considered age range ($45\text{-}100 \text{ mg day}^{-1}$) (18, 35). In contrast, the daily intake of the same volume of coffee with milk contributes with 40-85 mg of caffeine, reaching such limits.

If the Id:Ip (70:30 w/w) mixture is used in the making of MC with milk, with a daily intake of 400 mL, the daily caffeine does decrease to 15-17 mg. Thus, the use of such mixture seems to be the best combination to be used in the elaboration of MC with milk, for their safer doses of caffeine which allow greater consumption without increasing the risks.

Mineral content

The ash content of the MC made with the two studied species and their mixture, reached (on average) between 0.054 and 0.066 g/100 mL, while for milk it was an average of 0.7 g/100 mL and for sugar 0.046 g/10 g. Infusions prepared from *Ilex dumosa* and *I. paraguariensis*, as well as from the Id:Ip (70:30 w/w) mixture, presented high extractability of the mineral elements, oscillating between 60 and 70% of the mineral matter detected in the crude drug, in agreement with previous studies (31).

Table 4 (page 212) shows the mineral contribution of a 200 mL serving of MC obtained from *Ilex paraguariensis*, *I. dumosa* and their mixture (Id:Ip, 70:30 w/w), combined with whole milk and sugar.

No significant differences were found in the mineral composition of the 3 studied formulas. Taking into account one serving, some minerals (P, Zn and Cu) reached percentage values of DV enough for the MC+M+S to be considered as a source of those nutrients, since they can exceed 20% of the daily requirement.

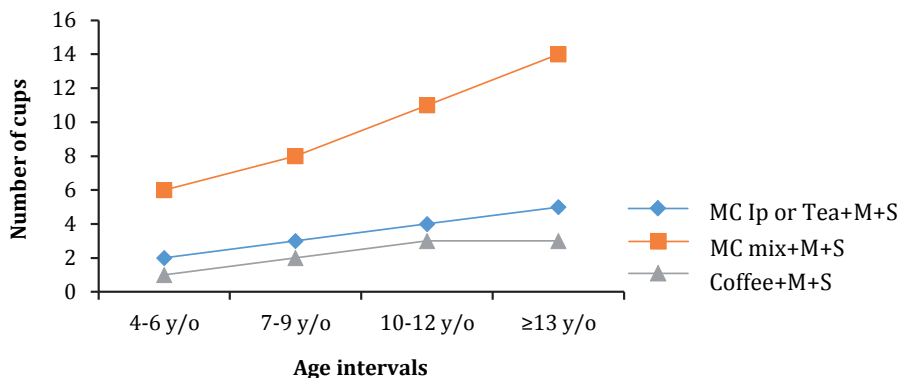
Table 3. Comparative number of cups to reach the caffeine limit of consumption of MC, Tea and Coffee (with milk and sugar) in 4-13 y/o children.

Tabla 3. Número comparativo de tazas para alcanzar el límite máximo de cafeína por consumo de MC, Té y café (con leche y azúcar) en escolares entre 4 y 13 años de edad.

Childrens age group	Daily caffeine intake limit ^(c)	N° of cups per day to reach the limit (a cup = 200 mL) ⁽¹⁾			
		MC _{Ip} +M+S 22±1.8 mg caffeine/cup ⁽²⁾	MC _{mix} +M+S 8±0.3 mg caffeine/cup ⁽²⁾	Tea+M+S 23±12 mg caffeine/cup ⁽³⁾	Coffee+M+S 57±20 mg caffeine/cup ⁽³⁾
4-6 y/o	≤45 mg	2	6	2	1
7-9 y/o	≤62.5 mg	3	8	3	≤2
10-12 y/o	≤85 mg	4	11	4	≤3
Adolescents ≥ 13 y/o (40-50kg bw) ^(d)	≤ 2.5 mg kg ⁻¹ bw (100-125 mg)	(4) 5 (6)	(12) 14.5 (16)	≤5	≤3

As in table 2 (page 208-209), (including serving size), plus: **y/o**: years old.- **bw**: body weight.- ^(c) according to Nawrot *et al.* (2003) and Health Canada (2012).- ^(d) statistical estimates of the "Looking at us: IMC Brigade" Project on the local school population from Educational Center N° 2, San Luis, Argentina (M.G. Del Vitto, pers. comm., August 2017).- ⁽¹⁾ in round numbers.- ⁽²⁾ in this work.- ⁽³⁾ according to Heckman *et al.* (2010), IFIC (2008) and CSPI (2014).

Como en tabla 2 (pág. 208-209), (incluyendo tamaño de porción), más: **y/o**: edad.- **bw**: peso corporal.- ^(c) según Nawrot *et al.* (2003) y Health Canada (2012).- ^(d) estimaciones estadísticas del Proyecto "Mirándonos: Brigada IMC" sobre la población escolar del Centro Educativo N° 2, San Luis, Argentina (M.G. Del Vitto, com. pers., agosto 2017).- ⁽¹⁾ en números redondos.- ⁽²⁾ en este trabajo.- ⁽³⁾ según Heckman *et al.* (2010), IFIC (2008) y CSPI (2014).



MC: "mate cocido".- **Ip:** *Ilex paraguariensis*.- **Id:** *I. dumosa*.- **mix:** Id+Ip 70:30 (w/w).- **M:** milk; **S:** sugar; **y/o:** years old.

MC: mate cocido.- **Ip:** *Ilex paraguariensis*.- **Id:** *I. dumosa*.- **mix:** mezcla de Id+Ip 70:30 (p/p).- **M:** leche; **S:** azúcar; **y/o:** años de edad.

Figure 1. Number of cups to reach the daily caffeine limit of consumption for MC, Tea and Coffee (with milk and sugar).

Figura 1. Número de tazas de MC, té y café (con leche y azúcar) para alcanzar el límite diario de cafeína.

Table 4. Mean contribution to the mineral daily intake per serving of "mate cocido" with milk and sugar, obtained from *Ilex paraguariensis*, *I. dumosa* and their mixture (1d:1p 70:30 w/w).

Table 4. Contribución promedio a la ingesta mineral diaria por porción de "mate cocido" con leche y azúcar, obtenida a partir de *Ilex paraguariensis*, *I. dumosa* y su mezcla (1d:1p 70:30 p/p).

Element	DRI mg day ⁻¹	MCIp+M+S		MCId+M+S		MCmix+M+S	
		mg/200 mL	%DV ⁽²⁾	mg/200 mL	%DV ⁽²⁾	mg/200 mL	%DV ⁽²⁾
Quantitative essential elements (required >100 mg day ⁻¹)							
K	3800-4500	157.8±26.2	4.08-4.84	153.7±22.4	2.9-4.63	155.2±18.2	3.04-4.56
Ca	1000-1300	121.69±19.8	7.84-14.15	122.1±23.2	7.6-14.5	121.85±23.6	7.56-14.54
Mg	130-240	15.40±2.3	5.45-13.61	15.55±3.5	5.02-14.6	15.45±1.9	5.64-13.34
P	500-1250	94.18±7.6	6.93-20.36	93.23±8.4	6.79-20.33	93.43±8.1	6.83-20.31
Na	1200-1500	49.28±7.2	2.8-4.71	48.97±6.8	2.81-4.64	49.07±7.8	2.75-4.74
Cl	1900-2300	142.4±25.3	5.09-8.83	140.74±21.7	5.17-8.54	141.24±22.7	5.15-8.63
Trace essential elements (required <100 mg day ⁻¹)							
Mn	1.5-1.9	1.38±0.32	55.8-113.3	1.613±0.24	72.3-123.5	1.603±0.26	70.68-124.2
Fe	8-10	0.305±0.05	2.55-4.44	0.295±0.06	2.35-4.43	0.296±0.08	2.16-4.7
Zn	5-8	0.82±0.22	7.5-20.8	0.84±0.18	8.25-20.4	0.828±0.14	8.6-19.36
Cu	0.44-0.7	0.08±0.01	10.0-20.45	0.085±0.01	10.7-21.6	0.083±0.02	9-23.41
Ultra-trace elements and others							
Al	2-10	0.07±0.02	0.5-4.5	0.07±0.01	0.6-4	0.07±0.01	0.6-4
Si	und.	1.65±0.54	und.	1.24±0.36	und.	1.363±0.42	und.
Ba	8-11	0.04±0.01	0.27-0.62	0.035±0.01	0.23-0.56	0.036±0.007	0.26-0.54
Sr	und.	0.12±0.02	und.	0.115±0.04	und.	0.117±0.03	und.
B	20	0.03±0.01	0.1-0.2	0.04±0.01	0.15-0.25	0.03±0.01	0.1-0.2

As in table 2 (page 208-209) and table 3 (page 211), (including serving size). **Note:** Ag, As, Cd, Co, Cr, Hg, Li, Mo, Ni, Pb, Sb, Se, Sn, Th, Ti, Tl, U and V were not detected or they were found below the instrumental limit of detection. DRIs according to FNB (2004).

Como en tabla 2 (pág. 208-209) y tabla 3 (pág. 211), (incluyendo tamaño de porción). - **Nota:** Ag, As, Cd, Co, Cr, Hg, Li, Mo, Ni, Pb, Sb, Se, Sn, Th, Ti, U y V no fueron detectadas o se hallaron por debajo del límite instrumental de detección. DRIs según FNB (2004).

In the case of Mn, it exceeds 50% of the DV and reaches up to 1.24 times the daily requirement, which is attributable to the contribution of both species of *Ilex*, in agreement with previous results (31).

For their part, a double daily portion of MC+M+S provides up to 40% of the DRIs of P, Zn and Cu, up to 29% of Ca, 27% of Mg, and 15% of Na and Cl, in addition to almost 10% of K, Fe and Al. It is noteworthy the contribution of Mn, since it far exceeds the DRI. These values of essential elements demonstrate the importance of this product in the student mineral nutrition (13).

In no case the analyzed elements exceeded the limits established by the international norms; the heavy metals did not make it to the infusion or milk, or were in a proportion well below the harmful values, which makes the product a safe food in terms of mineral elements.

Considering the nutritional goals set by diverse authors for school canteens in Argentina (5), the double serving of MC+M+S would provide approximately 40% of the required Ca, 35% of proteins, 30% of the energy value and vitamin A, 10% of Fe and 8% of vitamin C. These amounts are a bit higher than the calculated in this work, based on the DRIs, in regard to vitamin A, significantly higher in terms of energy value and vitamin C, almost triple in terms of Ca, double in Fe and significantly lower in proteins.

CONCLUSIONS

"Mate cocido" as a 1.5% infusion, does not represent *per se* a relevant food due to its dilution, except for some minerals and vitamins, and its content in chlorogenic acids and xanthines. However, mixed with

whole cow milk in a 50:50 proportion and sweetened with 10 g of refined sugar per cup ("mate cocido with milk"), it can become an important factor in the diet of students between 3 and 13 years old when their diet is supplemented with 1 to 2 daily servings of this food (serving size: a medium cup of 200 mL).

Considering a daily intake of 2 servings, except for niacin, folic acid and vitamins C, E and D, the rest of the nutritional parameters of the product exceed (sometimes by far) 20% of the DV, so that this food has to be considered as source of the following nutritional parameters and principles: energy value, proteins, carbohydrates, vitamins A, B₁, B₅ and B₆ ($\geq 20\%$ of the DRI); vitamins B₂ and B₁₂ ($\geq 50\%$ of the DRI); biotin ($\geq 100\%$ de la DRI), in addition to a low contribution of cholesterol. Regarding minerals, it has interesting contributions of P, Zn and Cu ($\leq 40\%$ of the DRI), Ca and Mg ($\leq 30\%$), Na and Cl ($\leq 15\%$) and K, Fe and Al (8-10%); with a noteworthy contribution of Mn, which doubles the DRI but still without reaching toxic values. In no case the heavy metals make it to the infusion or are well below the values considered as harmful.

The use of *Ilex paraguariensis* (Ip), *I. dumosa* (Id) or their commercial mixture (Id:Ip, 70:30 w/w) does not involve significant differences in nutritional parameters, both in the resulting infusions as well as in "MC with milk", except for the values of caffeine and chlorogenic acids. With Ip, "MC with milk" is close in caffeine values to "tea with milk", and contains from 1/2 to 1/3 of the caffeine found in "coffee with milk". "MC with milk" obtained from Id, contains 11 times less caffeine than "MC with milk" from Ip.

The mixture ensures the persistence of favorable organoleptic characters of *I. paraguayensis*, while the greater proportion of *I. dumosa* significantly lower the caffeine content in the final product (7-9 mg, that is, 2.75 times lower than the obtained from Ip and tea, and 10-20 times lower than in coffee), that is, within the recommended safety limits (40-100 mg day⁻¹) for the age range between 3-13 years.

The use of this mixture (Id:Ip, 70:30 w/w) in the preparation of "mate cocido" seems to be the best combination, since it maintains the product nutritional quality and palatability for most of the consumer

population, contributing to a better intellectual and physical performance of the students, without detrimental effects due to its lower doses of caffeine making it safer against the possible intake of multiple servings per day.

The studied food provides interesting nutritional values for some of the goals established as "reasonable" for some school canteens in Argentina (5), in particular in regard to energy, proteins, some vitamins (A and C), and minerals (Ca and Fe).

Finally, YM manufactured in "bags" of 3 g each, allows to properly dose the crude drug for consumption.

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Parameterization of the Hargreaves equation in the northern oasis of Mendoza, Argentina

Parametrización de la ecuación de Hargreaves en el oasis norte de Mendoza, Argentina

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ABSTRACT

In view of the water scarcity that affects the province of Mendoza, Argentina, information on reference crop evapotranspiration (ET_0) is crucial for irrigation scheduling. Data that are not generally available is required for the determination of ET_0 , with the Penman-Monteith FAO56 equation (PM). The Hargreaves equation (HG), which only requires air temperature data, represents an alternative to calculate ET_0 , after its local or regional calibration with PM. In this paper, the Hargreaves equation was calibrated locally by means of annual (C_a) and monthly ($C_{m,j}$) adjustment coefficients for the northern oasis of Mendoza. Also, a regionalisation of the C_a was performed considering environmental variables. The local adjustment with both coefficients made it possible to correct the positive bias that indicated an overestimation of HG with respect to PM in 12 meteorological stations. The mean value of the root mean square error decreased from 0.80 mm day^{-1} to 0.57 mm day^{-1} with the C_a adjustment coefficient, and to 0.55 mm day^{-1} with the $C_{m,j}$ adjustment coefficient, while the absolute error decreased from 0.63 to 0.42 and 0.39, respectively. Wind speed was the variable that best explained the regional variability of the C_a ($R^2 = 0.64$).

Keywords

reference evapotranspiration • irrigation programming • Hargreaves equation • water scarcity • adjustment coefficient

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RESUMEN

Ante la escasez hídrica atravesada por Mendoza, Argentina, es importante conocer la evapotranspiración referencia (ET_0) de los cultivos para programar el riego. Su cálculo, a partir de la ecuación de Penman-Monteith FAO56 (PM), requiere contar con datos no disponibles en general. La ecuación de Hargreaves (HG), que solo requiere datos de temperaturas del aire, representa una alternativa para calcular ET_0 , luego de su calibración local o regional con PM. En este trabajo se calibró localmente la ecuación de Hargreaves por medio de coeficientes de ajuste anuales (C_a) y mensuales ($C_{m,j}$) para el oasis norte de Mendoza. También, se llevó a cabo una regionalización del C_a considerando las variables ambientales. El ajuste local con ambos coeficientes logró eliminar el sesgo positivo que indicaba una sobrestimación de HG con respecto a PM en las 12 estaciones meteorológicas consideradas. El valor promedio del error cuadrático medio disminuyó de $0,80 \text{ mm día}^{-1}$ a $0,57 \text{ mm día}^{-1}$ con el ajuste con C_a y a $0,55 \text{ mm día}^{-1}$ con el uso de $C_{m,j}$, mientras que el error absoluto disminuyó de $0,63$ a $0,42$ y $0,39$ respectivamente. La velocidad del viento fue la variable que mejor explicó la variabilidad regional del C_a ($R^2 = 0,64$).

Palabras claves

evapotranspiración de referencia • programación de riegos • ecuación de Hargreaves
• escasez hídrica • coeficientes de ajuste

INTRODUCTION

Evapotranspiration from agricultural lands constitutes the greatest consumptive water use globally (7). Nearly 70% of the land in Argentina is arid (25) and the province of Mendoza, located in the central-western part of the country (32° and $37^\circ 35' \text{ S}$, $66^\circ 30'$ and $70^\circ 35' \text{ W}$), is one of the most desertified provinces with a mean annual rainfall of 200 mm. The rivers, which are fed by snowmelt from the Andes mountain range, and groundwater use have given rise to the so called "oasis" and a particular settlement pattern (11). The oasis, where 98% of the population is concentrated, occupies only 3% of the total land area. The available surface water resources are estimated at 6000 hm^3 per year (12) and groundwater withdrawals are about 250 and 600 hm^3 , depending on surface water availability

(16). The northern oasis of the province of Mendoza, irrigated by the Mendoza and Lower Tunuyán rivers, is the second largest irrigated area in Argentina (8). Climate change scenarios for the region (6) anticipate changes in climate as well as in local water availability. For the Andes mountain range, between 32° to 36° S parallels, the models developed by the Centro de Investigaciones del Mar y la Atmósfera (CIMA), based on scenario A2 (Scenario where effective mitigation measures or technological developments to reduce CO_2 emissions are not expected (IPCC, Synthesis Report 2001), predict a mean temperature increase of 1.25 to 1.5°C and a mean precipitation decrease of 100 mm for the period 2020-2030. A mean elevation of the 0°C isotherm of 150 to 130 metres with respect to the current

situation is also expected. Under this scenario, a decline in the winter snowpack and a progressive reduction of the ice cover are likely to occur. These changes will reduce water availability in the rivers running from the Andes mountain range and will consequently affect the irrigable lands. In this sense, since 2009 the province has been under a state of water emergency because the volume of water in the rivers of Mendoza is below the historical mean, principally due to little snowfall in the mountain range.

The water balance of the oasis shows a deficit of nearly $80 \text{ hm}^3 \text{ year}^{-1}$ for the Mendoza River ($1,041.46 \text{ hm}^3 \text{ year}^{-1}$ while water demand is $1,121.05 \text{ hm}^3 \text{ year}^{-1}$) and a notably increasing water deficit ($422 \text{ hm}^3 \text{ year}^{-1}$) for the Lower Tunuyán River (12) ($822 \text{ hm}^3 \text{ year}^{-1}$ while water demand is $1,244 \text{ hm}^3 \text{ year}^{-1}$). Water scarcity affects mainly the agricultural sector since it uses 92% of the water from the Mendoza and Tunuyán rivers.

In this sense, it is crucial to estimate crop water requirements accurately in order to: (i) plan irrigation management and ensure efficient water use, (ii) increase productivity and efficiency in water use, and (iii) reduce water and carbon footprints (22). Irrigation water requirements can be estimated on the basis of the reference evapotranspiration (ET_0) (2). Among the existing methods, the Food and Agriculture Organization of the United Nations (FAO) recommends to use the Penman-Monteith equation (FAO56-PM) as the standard method for estimating ET_0 (2).

The equation calls for information on temperature, relative humidity, solar radiation and wind speed, which is not always available. Other equations, such as the one proposed by Hargreaves (HG) and its modifications (13, 14, 15), basically

require data on air temperature that is available in most meteorological stations.

The HG equation has received considerable attention for its accurate ET_0 estimates in different climatic regions (2). However, it should be evaluated and calibrated to improve its application accuracy in each area (10, 24). For example, when the HG equation is applied in coastal regions, the ET_0 is usually underestimated while in interior regions it is overestimated (4, 10, 24). Moreover, when there is intense sensible heat advection on the surface, the HG tends to underestimate daily ET_0 values by 25% (5). Previous research has shown that the results obtained with the HG equation are very local and depend specifically on the location of each study (17). Therefore, it is often recommended to perform a local calibration of the HG equation (18) by adjusting the daily or monthly ET_0 values obtained with FAO56-PM (10, 18, 20, 24). The HG equation has been calibrated at international level in different locations with satisfactory results. In southeast China, Feng *et al.* (9) improved the adjustment of the HG equation with a relative root-mean-square error (RRMSE) and mean-absolute error (MAE) of 0.284 and $0.433 \text{ mm day}^{-1}$ for local calibration against 0.567 (RRMSE) and 0.959 (MAE) mm day^{-1} corresponding to the original HG model. In the semi-arid region of southeast Spain some authors have differentiated the adjustment of the equation for windy and non-windy zones (19) and between coastal and interior zones (10, 24). In this regard, Maestre-Valero *et al.* (2013) recommended a regionally calibrated HG equation for the calculation of long series of monthly ET_0 when they lacked complete data on temperature and air humidity, radiation or wind speed. In Argentina, Almorox *et al.* (2012) reported

that they had no meteorological data for calculating the ET_0 FAO56-PM in Coronel Dorrego (Buenos Aires) but since data on temperature was available, they recommended the HG method to estimate the ET_0 . Moreover, they concluded that the HG method, parameterized by means of a local coefficient calibration, is more in line with the FAO56-PM results, with an RMSE of 0.89 mm day^{-1} and an MBE of 0.27 mm day^{-1} .

Irrigators in the northern oasis of Mendoza are concerned about inaccurate meteorological information and the need for a service to estimate crop water requirements (1). In this context, in regions with scarce information on climate it is crucial to develop locally or regionally calibrated equations for accurate ET_0 estimates. Therefore, the objective of this work is to calibrate and validate the HG equation on a daily scale for the northern oasis of Mendoza in order to provide irrigators with a tool to estimate crop water requirements and contribute to the sustainability of the region's agricultural sector.

MATERIALS AND METHODS

Study area and climate data

This work was carried out in the northern oasis of Mendoza which lies between latitudes $32^{\circ}28'$ and $33^{\circ}23'$ S and longitudes $69^{\circ}03'$ and $67^{\circ}31'$ W. Daily meteorological information from 12 weather stations was used for the period 2008-2014 (2008-2011 for calibration and 2012-2014 for validation).

The principal characteristics of these stations are presented in table 1 and their location in the northern oasis is shown in figure 1 (page 221). Three of the stations belong to the Servicio Meteorológico Nacional (SMN) and the rest to the network of automatic meteorological stations of the provincial Dirección de Agricultura y Contingencias Climáticas (DACC). All stations provide hourly or tri-diurnal data on meteorological variables which the SMN and the DACC use to calculate daily values.

Table 1. Meteorological stations in the study area, location (latitude and longitude) and altitude.

Tabla 1. Estaciones meteorológicas del área de estudio, localización (latitud y longitud) y altitud.

Station (nomenclature)	Institution	Location	Altitude (m a. s. l.)
Chacras de Coria (Cha)	SMN	$32^{\circ}59' \text{ S } 68^{\circ}50' \text{ O}$	921
Mendoza Aero (MAero)	SMN	$32^{\circ}50' \text{ S } 68^{\circ}48' \text{ O}$	704
Mendoza Observatorio (MObs)	SMN	$32^{\circ}54' \text{ S } 68^{\circ}52' \text{ O}$	823
Jocolí (Joc)	DACC	$32^{\circ}35' \text{ S } 68^{\circ}35' \text{ O}$	590
Las Violetas (LasVio)	DACC	$32^{\circ}48' \text{ S } 68^{\circ}36' \text{ O}$	625
Tres Porteñas (3P)	DACC	$32^{\circ}54' \text{ S } 68^{\circ}23' \text{ O}$	627
Junín -(Jun)	DACC	$33^{\circ}07' \text{ S } 68^{\circ}29' \text{ O}$	667
Los Campamentos (LosCam)	DACC	$33^{\circ}15' \text{ S } 68^{\circ}27' \text{ O}$	661
El Mercado (ElMar)	DACC	$33^{\circ}05' \text{ S } 68^{\circ}13' \text{ O}$	622
Las Catitas (LasCat)	DACC	$33^{\circ}15' \text{ S } 68^{\circ}03' \text{ O}$	595
Russell (Rus)	DACC	$33^{\circ}01' \text{ S } 68^{\circ}45' \text{ O}$	790
Perdriel (Per)	DACC	$33^{\circ}07' \text{ S } 68^{\circ}54' \text{ O}$	981

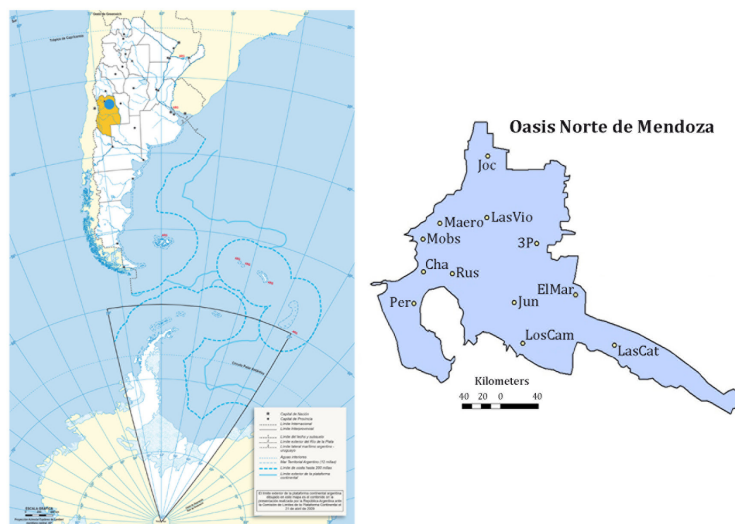


Figure 1. Location of the meteorological stations in the northern oasis of Mendoza, Argentina. The names of the stations are shown in table 1 (page 220).

Figura 1. Localización de las estaciones meteorológicas en el oasis norte de Mendoza, Argentina. Los nombres de las estaciones se pueden ver en la tabla 1 (pág. 220).

FAO-56 Penman-Monteith Equation

Due to the lack of direct measurements of real evapotranspiration, either by lysimeter or by soil water balance, the reference value of ET_0 is the one recommended in FAO-56 (2) (ET_0PM):

$$ET_0PM = \frac{0.408(R_n - G) + \gamma \left[\frac{900}{(T_{med} + 273)} \right] V_2 (e_s - e_a)}{A + \gamma(1 + 0.34V_2)} \quad (1)$$

where:

ET_0PM = reference evapotranspiration (mm day⁻¹)

R_n = net radiation on crop surface (MJ m⁻² day⁻¹)

G = ground heat flux (MJ m⁻² day⁻¹)

T = mean air temperature at a height of 2 m (°C)

V_2 = wind speed at a height of 2 m (m s⁻¹)

e_s = equilibrium vapour pressure or saturated vapour pressure at air temperature (kPa)

e_a = real air vapour pressure (kPa)

$e_s - e_a$ = air vapour pressure deficit (kPa)

Δ = slope of the equilibrium vapour pressure curve (kPa °C⁻¹)

γ = psychrometric constant (kPa °C⁻¹).

These variables and physical parameters were determined following the method outlined in FAO-56, which provided daily estimates of reference evapotranspiration in the 12 stations (ET_0PM). They were used to calibrate (period 2008-2011) and validate (period 2012-2014) the different adjustment coefficients proposed for the Hargreaves equation.

Hargreaves Equation

The Hargreaves-Samani equation (1985) was used to determine ET_0 (ET_0HG) as a function of the extraterrestrial radiation (R_a ; mm day⁻¹), and the mean (T_{med} ; °C), minimum (T_{min} ; °C) and maximum (T_{max} ; °C) air temperature.

$$ET_0HG = C R_a (T_{med} + 17.78)(T_{max} + T_{min})^{0.5} \quad (2)$$

where:

$C = 0.0023$, represents the Hargreaves coefficient.

Local calibration of the Hargreaves equation

Calibration by means of the annual adjustment coefficient

A calibration of the HG method was carried out using the C_a annual adjustment coefficient for each station.

The adjustment was carried out by calculating forced-origin linear regressions from the daily calculated values of ET_0PM and $ET_0HG/0.0023$ in the period 2008-2011. The values of C_a substituted 0.0023 in (2), thus the HG equation calibrated with an annual coefficient (ET_0HG_a) was obtained.

Calibration by means of monthly adjustment coefficients

The calibration was also performed by means of monthly $C_{m,j}$ adjustment coefficients for each station obtained by forced-origin linear regressions from daily calculated values of ET_0PMM,j and $ET_0HGm,j/0.0023$ in the period 2008-2011, where ET_0PMM,j and ET_0HGm,j are the daily ET_0 values for the month j by PM and HG, respectively.

Adjustment validation of the Hargreaves equation

The equations calibrated with the C_a and $C_{m,j}$ adjustment coefficients were validated using the 2012-2014 data series for each of the stations (table 1, page 220) that were not previously used for the calibration. The results obtained with the original HG equation (ET_0HG) were statistically compared between their annual (ET_0HG_a) and monthly ($ET_0HG_{m,j}$) calibrations -expected values- and those calculated from the ET_0PM -observed values- by, (i) the determination coefficient, R^2 ; (ii) the mean bias error, MBE; (iii) the root-mean-square error, RMSE, and (iv) the absolute error, AE:

$$R^2 = \frac{\sigma^2 xy}{\sigma^2 x \sigma^2 y} \quad (3)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_i - x_i)^2}{n}} \quad (4)$$

$$MBE = \frac{\sum_{i=1}^n (y_i - x_i)}{n} \quad (5)$$

$$AE = \frac{\sum_{i=1}^n |y_i - x_i|}{n} \quad (6)$$

where:

n = number of data

y = the expected value of ET_0 (ET_0HG , ET_0HG_a or $ET_0HG_{m,j}$)

x = the observed value of ET_0 (ET_0PM)

σ_{xy} = the covariance of x and y

σ_x = the typical deviation of x

σ_y = the typical deviation of y

Regional calibration of the Hargreaves coefficient

In order to determine one or more variables that explain the variability of C_a among the stations, a sensitivity analysis was carried out for different environmental variables using linear regressions. The mean annual variables that were analyzed were: latitude, altitude -station's altitude-, T_{max} , T_{min} , T_{med} , P (atmospheric pressure), V_2 , R_a , R_n , HR (relative humidity), $\Delta T (T_{max} - T_{min})$ and $\Delta T / T_{med}$. For each station, a matrix of correlations among these variables and C_a was calculated to determine which variables exert the greatest impact to explain the variance at a 99% confidence level.

The determination coefficient R^2 was higher than 0.80 for all the stations, with a mean of 0.91. The statistical analyzes (RMSE, MBE and AE) showed significant errors. Considering all stations, the RMSE varied between 1.034 and 0.49 mm day⁻¹, with a mean of 0.80 ± 0.18 mm day⁻¹, the MBE varied between 0.698 and 0.077 mm day⁻¹ with a mean of 0.41 ± 0.23 mm day⁻¹ and the AE varied between 0.802 and 0.375 mm day⁻¹ with a mean of 0.63 ± 0.14 mm day⁻¹.

The mean values of the MBE indicated that ET_0HG overestimated ET_0PM in all stations. These results are similar to those that Gavilán *et al.* (2006) obtained for interior zones in southern Spain, where the climate is semi-arid and local calibrations of the HG equation are required.

RESULTS AND DISCUSSION

Application of the original Hargreaves equation

The results of the statistical comparison between ET_0PM and ET_0HG are shown in table 2.

Local calibration using annual adjustment coefficients (C_a)

According to other similar studies performed in arid and semi-arid regions (10, 18, 22), annual coefficients for all stations were lower than the original HG coefficient (0.0023).

Table 2. Statistical results among daily ET_0 values with the Penman-Monteith (ET_0PM) and original Hargreaves (ET_0HG) equations.

Tabla 2. Resultados estadísticos entre valores diarios de ET_0 con Penman-Monteith (ET_0PM) y Hargreaves original (ET_0HG).

Station	R ²	RMSE	MBE	AE
Chacras de Coria	0.88	1.034	0.686	0.802
Mendoza Aero	0.97	0.738	0.619	0.633
Mendoza Observatorio	0.80	0.998	0.173	0.716
Jocolí	0.88	0.898	0.460	0.656
Las Violetas	0.98	0.830	0.698	0.712
Tres Porteñas	0.96	0.494	0.189	0.375
Junín	0.98	0.811	0.686	0.704
Los Campamentos	0.84	0.903	0.130	0.695
El Mercado	0.91	0.698	0.411	0.570
Las Catitas	0.94	0.485	0.077	0.376
Russell	0.94	0.706	0.427	0.536
Perdriel	0.83	0.980	0.379	0.801
Mean	0.91 ± 0.06	0.80 ± 0.18	0.41 ± 0.23	0.63 ± 0.14

C_a presented a rather stable value ranging between 0.0019 (Cha, LosCam, LasCat) and 0.0022 (Joc, LasVio, Maero, Per, 3P) and a mean value of 0.0021. Figure 2, shows the spatial variation of C_a in the Northern oasis of Mendoza, although no clear spatial behavioural pattern or possible relationship with other geographic variables such as altitude or latitude can be identified.

The results obtained agree with those presented by Martinez Cob *et al.* (2004) for non-windy semi-arid regions in Spain, where they proposed a coefficient of 0.0020, or with those obtained by Vanderlinden *et al.* (2004) in interior areas of southern Spain where the annual mean of the adjustment coefficient was 0.0022.

Validation of the use of an annual coefficient

The determination coefficient (R^2) between ET_0HG_a and ET_0PM was practically identical to that obtained with the original HG, with no appreciable differences in any of the stations (table 3, page 225).

The RMSE improved considerably, ranging between 0.96 and 0.26, with a mean of 0.57 ± 0.24 mm day⁻¹. The adjustment bias was almost completely eliminated, with the MBE ranging between 0.17 and -0.03, with a mean of 0.04 ± 0.07 mm day⁻¹.

Finally, the AE also improved considerably when compared to that obtained with the original HG, showing values between 0.69 and 0.21, with a mean of 0.42 ± 0.18 mm day⁻¹.

The MBE values, close to zero, demonstrated that the use of an equation calibrated with an annual coefficient eliminated the systematic overestimation produced by the original HG in calculating the daily ET_0 .

Local calibration by means of monthly adjustment coefficients ($C_{m,j}$)

Monthly coefficients showed a similar pattern throughout the 12 stations (figure 3, page 225-226), with slight decreases in value during the autumn months and slight increases at the end of winter and early spring.

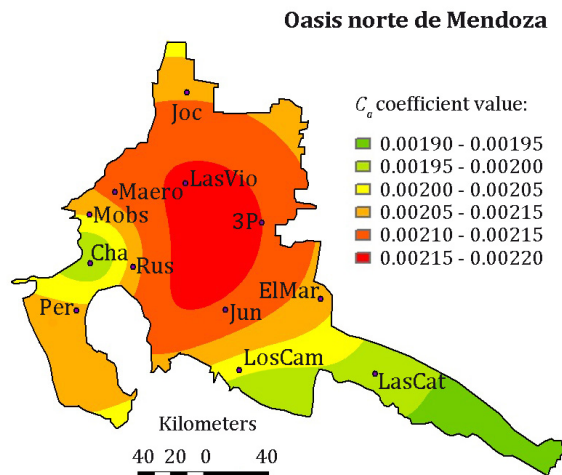


Figure 2. Map of annual coefficient values in the northern oasis of Mendoza.

Figura 2. Mapa de valores de coeficiente anual en el oasis norte de Mendoza.

Table 3. Statistical results among daily ET_0 values with Penman-Monteith (ET_{0PM}) and Hargreaves calibrated with an annual coefficient (ET_{0HG_a}).

Tabla 3. Resultados estadísticos entre valores diarios de ET_0 con Penman-Monteith (ET_{0PM}) y Hargreaves calibrada con coeficiente anual (ET_{0HG_a}).

ET_{0HG_a}	R^2	RMSE	MBE	AE
Chacras de Coria	0.88	0.624	0.024	0.448
Mendoza Aero	0.97	0.314	-0.030	0.247
Mendoza Observatorio	0.80	0.959	0.011	0.673
Jocolí	0.88	0.720	0.166	0.512
Las Violetas	0.98	0.300	0.019	0.229
Tres Porteñas	0.96	0.448	-0.024	0.310
Junín	0.98	0.260	0.032	0.206
Los Campamentos	0.84	0.895	-0.015	0.662
El Mercado	0.91	0.497	-0.004	0.369
Las Catitas	0.94	0.469	-0.007	0.353
Russell	0.94	0.501	0.126	0.358
Perdriel	0.83	0.885	0.143	0.688
Mean	0.91 ± 0.06	0.57 ± 0.24	0.04 ± 0.07	0.42 ± 0.18

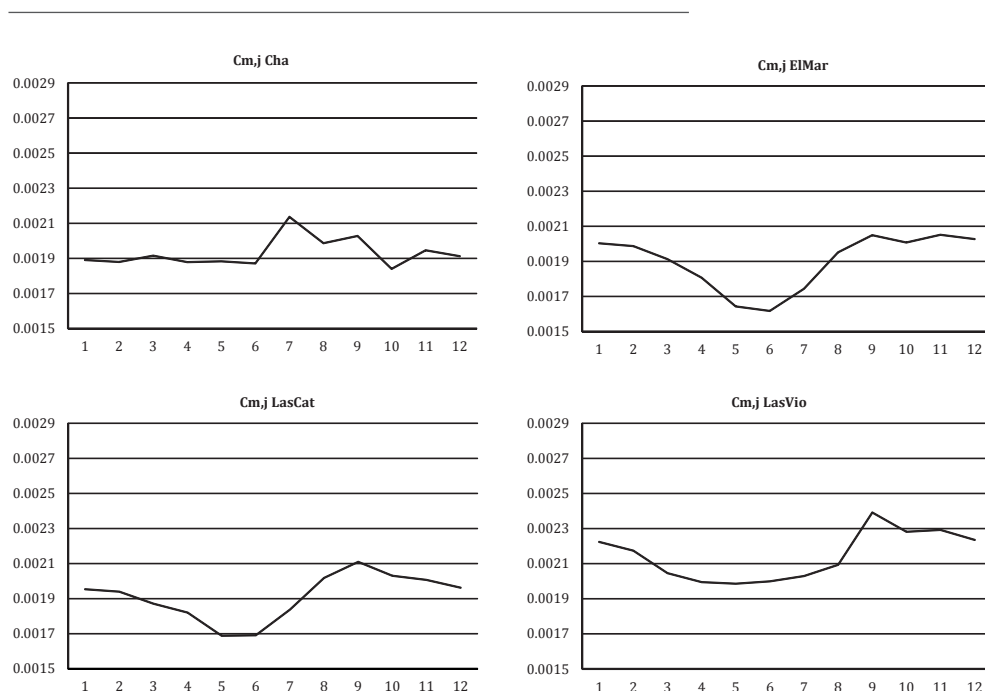


Figure 3. Evolution of monthly coefficients ($C_{m,j}$).
Figura 3. Evolución de coeficientes mensuales ($C_{m,j}$).

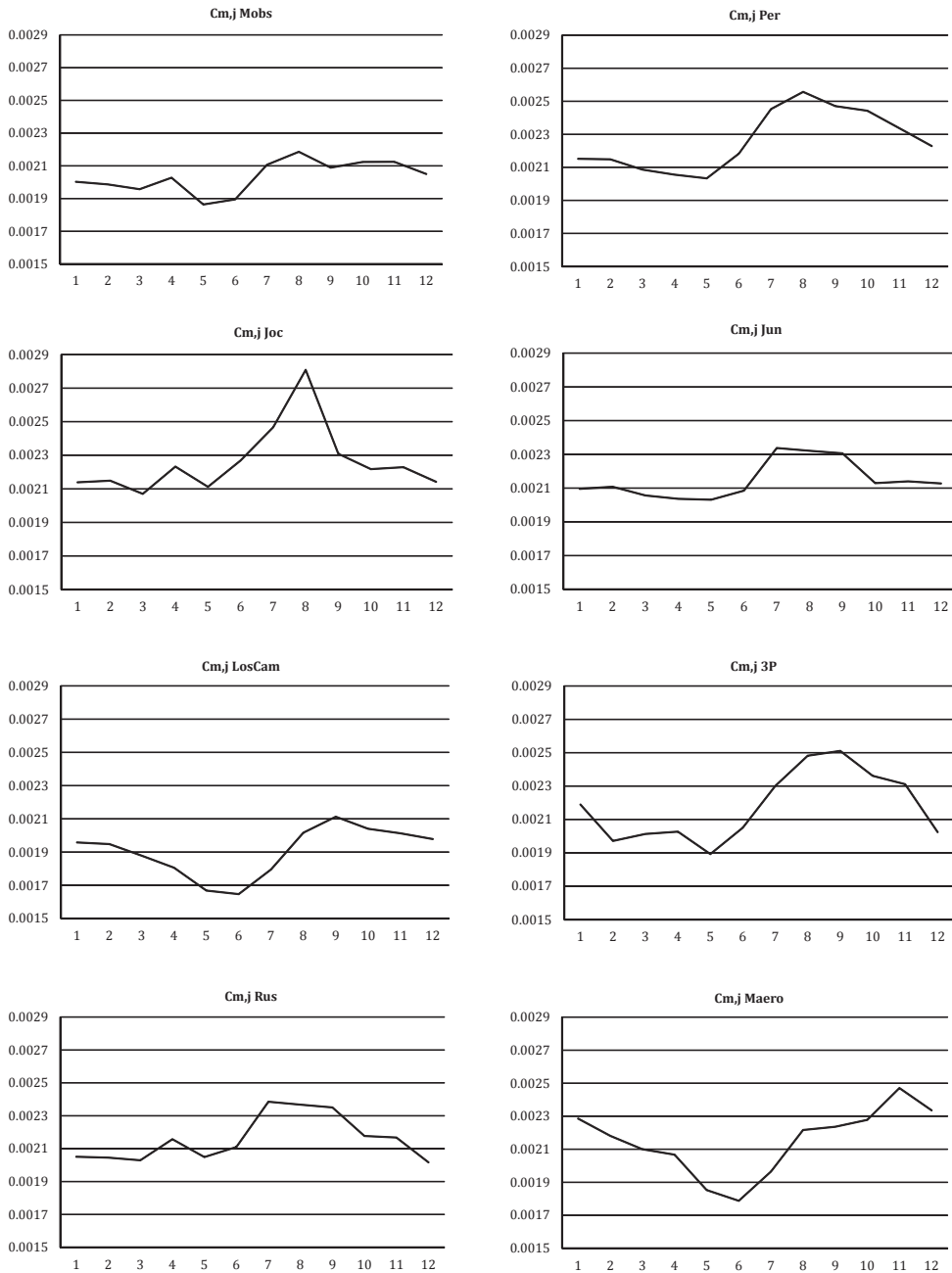


Figure 3 (cont.). Evolution of monthly coefficients ($C_{m,j}$).
Figura 3 (cont.). Evolución de coeficientes mensuales ($C_{m,j}$).

This variation was similar to the intensity of the Zonda wind (21), whose variability throughout the year presents the same behaviour.

In general, the $C_{m,j}$ were also lower than the original HG value (0.0023), with the exceptions of the Joc, Per and 3P stations where it was exceeded by the end of winter when the Zonda wind reaches its maximum intensity.

Validation of the use of monthly coefficients

Calibration by means of monthly coefficients virtually reduced the adjustment bias (mean of the MBE of 0.04 ± 0.07) eliminating the systematic overestimation produced by the original HG. Additionally, the values of RMSE and AE decreased slightly with respect to the calibration with the annual coefficient (table 4).

The improvement in RMSE and AE is most significant in those stations with greater monthly $C_{m,j}$ variability, as is the case of the *El Mercado* and *Perdriel* stations.

Regional calibration by means of annual coefficients

An assessment of the correlation performed among the different environmental variables and the annual adjustment coefficient (C_a) showed that wind speed is the variable that best explains the higher percentage of adjustment variance ($R^2 = 0.64$; figure 4, page 228). Other authors also found a good relationship between wind speed and the HG coefficient. For a semi-arid region in southern Spain Martínez-Cob and Tejero (2004) used annual mean wind speed as the parameter defining calibration groups of the HG equation and recommended not to perform a calibration when the wind speed was over 2 m s^{-1} due to the good performance of HG with respect to PM. Moreover, they suggested using a coefficient of 0.0020 rather than 0.0023 for non-windy locations, where ET_0 HG overestimated ET_0 PM with a mean error of 14 to 20%.

Table 4. Statistical results of daily ET_0 values with Penman-Monteith (ET_0 PM) and Hargreaves calibrated with monthly coefficients (ET_0 HG $C_{m,j}$).

Tabla 4. Resultados estadísticos entre valores diarios de ET_0 con Penman-Monteith (ET_0 PM) y Hargreaves calibrada con coeficientes mensuales (ET_0 HG $C_{m,j}$).

Station	R ²	RMSE	MBE	AE
Chacras de Coria	0.87	0.622	0.026	0.447
Mendoza	0.98	0.274	-0.023	0.186
Mendoza Observatorio	0.80	0.942	0.022	0.646
Jocolí	0.88	0.700	0.168	0.495
Las Violetas	0.98	0.267	0.025	0.187
Tres Porteñas	0.96	0.408	-0.023	0.268
Junín	0.98	0.255	0.033	0.189
Los Campamentos	0.85	0.882	-0.007	0.635
El Mercado	0.92	0.483	-0.003	0.355
Las Catitas	0.95	0.442	-0.004	0.316
Russell	0.94	0.470	0.126	0.326
Perdriel	0.83	0.882	0.141	0.677
Mean	0.91 ± 0.06	0.55 ± 0.25	0.04 ± 0.07	0.39 ± 0.18

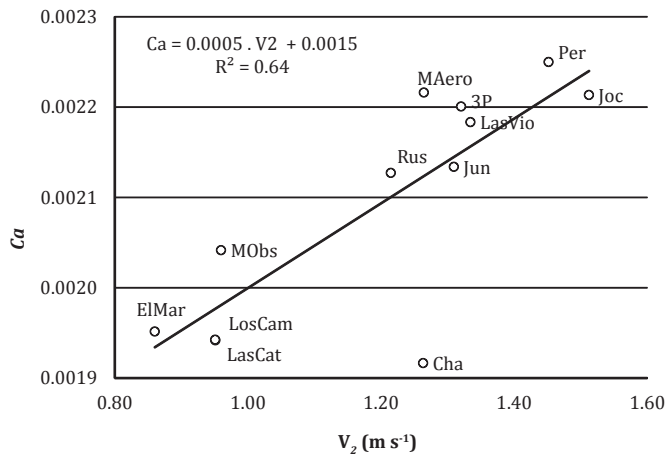


Figure 4. Dispersion between V_2 (m s⁻¹), and C_a (dimensionless) values of the ET_0HG_a equation.

Figura 4. Dispersión entre valores de V_2 (m s⁻¹), y C_a (adimensional) de la ecuación de ET_0HG_a .

For the Oasis of Mendoza the mean annual wind speed at 2 m is low in all stations (less than 1.5 m s⁻¹) and ET_0HG performed better in those stations with higher wind strengths (near 1.5 m s⁻¹).

The mean value of the annual HG adjustment coefficient for locations with weak winds was 0.0020 for the Oasis (figure 2, page 224). Gavilán *et al.* (2006) found overestimations of ET_0HG over ET_0PM in interior areas of Spain. They concluded that V_2 and ΔT exerted a significant influence on the precision of the HG equation and classified overestimated and underestimated areas through a combination of these two variables.

The regions with V_2 below 1.5 m s⁻¹ and with ΔT above 12 °C overestimated the daily ET_0 value that was calculated with ET_0HG ; a coefficient of 0.0021 must be used to improve the estimates. This

example explains the results obtained in the northern oasis of Mendoza, where the annual thermal amplitude is even greater ($\Delta T > 15^\circ\text{C}$) in all locations due to desert and continentality conditions.

Finally, Shahidian *et al.* (2013) analysed different environmental variables to perform the calibration of ET_0HG by testing different models under different climate scenarios. They concluded that wind speed was the most important parameter to improve ET_0HG estimates. However, in the northern oasis of Mendoza wind speed values are not usually available when the ET_0HG equation can be useful. So, from a practical point of view, regional calibration of the HG equation is less interesting than local calibration.

CONCLUSIONS

On the basis of its original formulation, the Hargreaves equation is not suitable for irrigation scheduling because it offers systematic and considerable overestimates for ET_0 calculation in the northern oasis of Mendoza on a daily scale. Nevertheless, the accuracy of the equation for calculating ET_0 on a daily scale improves with the use of an annual adjustment coefficient and monthly calibration coefficients in all stations included in this study. The differences between the annual adjustment coefficient and monthly calibration coefficients are negligible, though a slight decrease in errors was detected in all stations when monthly calibration coefficients are used. Since the information required for both is the same, it is recommended to use the monthly calibration coefficient, although both methodologies can be considered equally valid.

With regard to the regionalisation of the Hargreaves equation in the northern oasis

of Mendoza, the variability of environmental patterns given the great continentality and desert conditions are scant. This prevented to explain the spatial variability of the annual adjustment coefficient C_a as a function of variables that are readily available in the zone.

The main explanatory variable has been wind speed ($R^2 = 0.64$), which is of little use given that this information is not available in most of the stations of the northern oasis of Mendoza (which is why alternative methods to the Penman-Monteith are necessary, as proposed here).

Therefore, it can be concluded that when only air temperature data are available either of the two methods for local calibration of the HG equation presented in this study is suitable for estimating crop water requirements on a daily scale in the northern oasis of Mendoza.

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Empresas agroalimentarias y adopción de las TIC: el caso del sector del aceite de oliva ecológico en España

Agricultural companies and adoption of ICT: the case of the ecological olive oil sector in Spain

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RESUMEN

El mercado de productos agroalimentarios ecológicos en España se enfrenta a un exceso de oferta provocado por la existencia de una de las mayores ofertas de estos productos a nivel mundial y, al mismo tiempo, de una demanda interna prácticamente inexistente. En este contexto, las TIC (Tecnologías de la Información y Comunicación), tienen el potencial de dar respuesta a estos problemas comerciales, por los múltiples beneficios y fortalezas que se derivan de su aplicación para fines empresariales. De acuerdo con ello, y tomando como objeto de estudio uno de los principales sectores agroalimentarios ecológicos en España, esto es, el del aceite de oliva, el presente trabajo ha tratado de analizar si aquellas características que la literatura señala como relevantes para explicar la adopción y uso eficiente de las TIC en la empresa, están efectivamente presentes entre las empresas de dicho sector. A través de un trabajo de campo dirigido a una muestra representativa del citado sector, se han podido detectar importantes debilidades estructurales y organizativas que obstaculizan la adopción y aprovechamiento de las TIC en su actividad empresarial.

Palabras claves

TIC • productos ecológicos • aceite de oliva • factores organizacionales • planificación de innovaciones

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ABSTRACT

The organic agri-food market in Spain faces an oversupply situation as a result of having one of the largest outputs of these products in the world, together with practically non-existent internal demand. In view of this, ICT (Information and Communication Technologies), has potential for providing an answer to the sector's commercial problems, owing to the many benefits and strengths arising from its application for business purposes. Accordingly, the present study addresses one of the main organic agri-food sectors in Spain, olive oil, attempting to ascertain whether the characteristics that have been pointed to in the literature as important for explaining the adoption and efficient use of ICT by businesses are in fact present in firms in this sector. Through a field study of a representative sample of the organic olive oil sector, it has identified important structural and organizational weaknesses that hinder these firms' adoption and use of ICT in their business activities.

Keywords

ICT • organic products • olive oil • organizational factors • planning innovations

INTRODUCCIÓN

El mercado de productos ecológicos (nacional e internacional) ha mostrado durante la última década un continuo crecimiento, poniendo de relieve su importancia presente y futura en la economía global (78). En este contexto, España ocupa una posición privilegiada en dotación de factores de producción, situándose como líder en Europa y en un quinto puesto a nivel mundial en superficie productiva (52, 77).

Hay que destacar que, dentro de la diversidad de cultivos de producción ecológica, la producción de aceitunas para la elaboración de aceite de oliva ocupa un papel protagonista, acaparando en torno al 46,5% de la superficie total dedicada a producción ecológica (52). Este potencial contrasta, sin embargo, con el reducido consumo interior de productos ecológicos en España, que asciende a tan solo 21 euros per cápita y año, lo que representa un 1 por ciento del total de gasto alimentario nacional (52). Esta situación de

exceso de oferta obliga a destinar el grueso de la producción ecológica a los mercados exteriores, lo que ha llevado a España a situarse como el tercer país exportador del mundo (77), importancia que también pone de relieve la PAC (Política Agraria Común) dentro del papel determinante del cuidado al medio ambiente (27).

Como principales factores explicativos de este exceso de oferta en el mercado interno se encuentran: la falta de promoción y apoyo a las nuevas tecnologías; la confusión y desinformación sobre las características de estos productos por parte de los consumidores; la dispersión y escasez de puntos de venta; y, especialmente, un excesivo diferencial de precio entre los productos ecológicos y los convencionales (30, 55, 57, 63, 65, 72, 73).

Entre las soluciones al problema comercial se encuentra el uso de las Tecnologías de la Información y la

Comunicación (en adelante TIC) basadas en Internet, especialmente los sitios web y las redes sociales online (55).

La literatura destaca el importante rol que pueden desempeñar estas plataformas en las organizaciones, por los múltiples beneficios y fortalezas que de ellas se derivan (11, 48, 76). Algunos de ellos se deben a su potencial informativo y comercial, capaz de cambiar la actitud reacia presente en el consumo de productos ecológicos y servir de acicate para desarrollar ventajas competitivas en las organizaciones (35).

Otros beneficios se asocian a su potencial para restar importancia a las distancias físicas que separan a la oferta de la demanda (75), facilitando el acceso a los mercados internacionales y la búsqueda de socios y soluciones comerciales. Esta ventaja es especialmente atractiva en el caso de las Pymes (Pequeñas y Medianas Empresas), la mayoría de las empresas que conforman el sector agroalimentario ecológico español, debido a sus limitados recursos financieros y humanos (62).

Todas las ventajas que conllevan el uso empresarial de las TIC han permitido a las empresas reducir los costes de transacción y han logrado modificar sustancialmente las reglas de la competencia (42). En esta línea, son numerosos los investigadores que han tratado de identificar cuáles son los factores explicativos de la adopción de una nueva tecnología o herramienta TIC por parte de la empresa (13, 21, 26, 34, 39, 49, 50, 56, 60, 70).

De acuerdo con lo anterior, el objetivo de este trabajo consiste en analizar si aquellas características que la literatura señala como relevantes para explicar la adopción de las TIC en la empresa, están efectivamente presentes en las empresas agroalimentarias de producción ecológica que han optado por la utilización de la

web como canal de comercialización de sus productos. De esta manera el presente estudio pretende contribuir a señalar las posibles deficiencias estructurales del sector que están impidiendo el avance comercial de este, a través de la utilización de las tecnologías de la información y comunicación.

Para abordar dicho objetivo, el trabajo se ha estructurado de la siguiente forma: tras la presente introducción, en un segundo apartado se realiza una revisión de la literatura para identificar los factores determinantes de la adopción de las TIC en las empresas. En un tercer apartado se explica la metodología empleada para el estudio. En un cuarto apartado se analiza en qué medida cada uno de los factores explicativos que la literatura subraya como determinantes de la adopción de las TIC en las empresas, están presentes entre las empresas agroalimentarias ecológicas españolas. Por último, en el cuarto apartado, se plantean las principales conclusiones obtenidas.

Factores explicativos de la adopción de las TIC en la empresa: una revisión de la literatura

El desarrollo rural ha sido el centro de atención de numerosas corrientes ideológicas (17).

Entre estas ideologías destaca el enfoque de modernización, con el objetivo de dar respuesta a los problemas rurales a través de la tecnología (36). En este sentido, Perkins y Witt (1961) indicaba la importancia de la incorporación de innovaciones tecnológicas en el apartado comercial de la agricultura, siendo indispensable para la mejora de competitividad del sector (8).

Según Castro y Rajadel (2015), la tecnología y la innovación apoyan, dirigen y estimulan el desarrollo local,

favoreciendo la productividad empresarial, la competitividad y el desarrollo social, económico e intelectual.

De acuerdo con lo anterior, numerosos investigadores han tratado de identificar cuáles son los factores explicativos de la adopción de una nueva tecnología o herramienta TIC por parte de la empresa.

En la siguiente tabla se resumen aquellos que ha sido objeto de un estudio más profundo en la literatura (tabla 1):

A continuación se profundiza en el efecto que cada uno de dichos factores tiene sobre la adopción de las TIC por parte de las empresas.

Antigüedad de la empresa

Un factor estudiado con frecuencia en la literatura al objeto de identificar su posible efecto sobre la actitud innovadora de la empresa es la antigüedad. Pese a ello, los estudios no son concluyentes sobre

la dirección entre antigüedad y actitud innovadora. En este sentido, los años de funcionamiento de la organización facilitan la adopción de los avances tecnológicos, con el fin de que las empresas estén en sintonía con el entorno (20). Asimismo, Chesbrough (2005), señala que el contacto continuado a lo largo del tiempo con otras entidades estimula la propensión a innovar.

De acuerdo con estos autores, Goode y Stevens (2000) exponen que la antigüedad supone una experiencia añadida, determinante a la hora de no dejar escapar las oportunidades del mercado. No obstante, desde otros estudios e investigaciones se manifiesta justamente lo contrario.

Según Pavitt (2003), las empresas de mayor antigüedad pueden presentar hándicaps añadidos para la incorporación de la innovación, derivados de posibles automatismos organizativos y comerciales adquiridos con el paso del tiempo.

Tabla 1. Factores estudiados en la literatura sobre la adopción de TIC.

Table 1. Studied factors in the literature on the adoption of ICT.

Factores	Fuentes bibliográficas
Tamaño empresarial	Tether (2005), Kim y Jee (2007) y Medina (2014).
Antigüedad de la empresa	Pavitt (2003), Hollenstein (2004), Chesbrough (2005), Díaz <i>et al.</i> (2006), Cucculelli (2014).
Existencia de presiones	Levy <i>et al.</i> (2005), Chong (2006), Dembla <i>et al.</i> (2007), Kim y Jee (2007), Alderete (2010), Moral <i>et al.</i> (2014).
Características de la dirección	Nguyen y Barret (2006), González y Peña (2007), Manchese y Jones (2011), Benito y Platero (2012), De Lucas (2015).
Personal disponible	Bruque (2002), Chun (2003), Hollenstein (2004), García <i>et al.</i> (2008), Medina (2014).
Beneficios percibidos	Doherty <i>et al.</i> (2003), Levy <i>et al.</i> (2005), González y Peña (2007), García <i>et al.</i> (2008).
Apoyo de la dirección y planificación de innovaciones	Gupta y Singhal (1993), Sieber <i>et al.</i> (2006), Medina (2014), Moral <i>et al.</i> (2015).

Fuente: elaboración propia. / Source: self made.

Por tanto, las organizaciones más antiguas pueden ser más reticentes a modificar su actividad y su forma de actuar, adquiriendo una mayor rigidez para incorporar las innovaciones del mercado (16). En este debate se observa una tercera postura, la cual señala que la adopción de la innovación dependerá del sector de actividad al que pertenezca la empresa y de sus propias características. En esta línea, Frenkel *et al.* (2001) afirman que las empresas jóvenes serán más proclives a la innovación en aquellos sectores vinculados con la alta tecnología. En cómputo, puede afirmarse que la antigüedad puede actuar como facilitador o como inhibidor, para la adopción de TIC en las empresas, ya que una mayor experiencia por la adopción de otras tecnologías puede compensarse por las reticencias al cambio que suelen tener estas (33).

Tamaño de la empresa

Según Rothwell (1983), el tamaño también es otro de los principales factores que afectan a la actitud innovadora de las empresas, siendo las Pymes, las entidades más propensas a adoptar las innovaciones organizativas, mientras que las grandes organizaciones lo son para las innovaciones tecnológicas (69). Entre las razones señaladas en la literatura que explican la menor propensión de las Pymes a adoptar las TIC se encuentran: la falta de recursos financieros. Respecto de los recursos financieros, autores como Benito y Platero (2012) sostienen que la innovación no conlleva, en todos los casos, un aumento de los costes de la empresa, debido a que numerosas herramientas TIC son de carácter gratuito.

No obstante, sí acarrearían un coste temporal y de personal, si se quieren adoptar con unas mínimas garantías de éxito; la falta de personal; la falta de tiempo

y la falta de conocimientos tecnológicos (49). Sin embargo, Kim y Jee (2007) revelaban un cambio de esta tendencia, al ser las Pymes las que apuestan de forma más activa por las TIC.

Recursos humanos

Los recursos humanos tienen una importancia indudable en la organización, así como en la nueva economía del conocimiento (45), siendo aún más determinantes en sectores con un alto grado de competitividad, como es el caso del sector de los productos ecológicos (49). De hecho, esta variable es una de las magnitudes más importantes para la adopción de las TIC en la empresa, dado el binomio imprescindible entre tecnología y recursos humanos (7, 38). Así, numerosos autores afirman que el personal presente en la organización y su formación son factores determinantes para la adopción de innovaciones (14, 15, 33). En esta línea, Carmona *et al.* (2000) señalan que es importante contar con recursos humanos notables y distintivos, para crear valor en la empresa y obtener un mejor rendimiento de las TIC. Del mismo modo, es necesario realizar programas de formación continuados en el tiempo (24, 46), para mejorar la competencia tecnológica y la receptividad de las nuevas tecnologías.

Máximo responsable de la administración

El puesto de máximo dirigente de la organización es un factor clave en el devenir de esta, razón por la que un gran número de estudios se han centrado en analizar las características de la persona que ocupa esta posición para establecer relaciones con la situación y visión de la empresa.

Según estos estudios, el papel del director general de la empresa es fundamental en la adopción de las nuevas TIC (50), tanto para la adopción de

innovaciones tecnológicas sugeridas directamente desde la dirección, como para el apoyo de otras promovidas por el resto de empleados. Así, puede afirmarse que determinadas características del máximo responsable facilitan la innovación (29) y permiten a la empresa su adaptación al contexto social y tecnológico actual (18), relación positiva que se ve potenciada en el caso de las Pymes (49). Entre dichas características, a continuación se van a abordar tres: edad, formación académica y conocimientos en TIC:

- Edad

La edad del máximo responsable de la administración de la empresa se ha relacionado frecuentemente con la propensión innovadora de este y, por tanto, con la adopción de las TIC en la empresa que administra y dirige.

Según Benito y Platero (2012), esta característica es aún más importante en las microempresas, que representan el 56,57 por 100 de las entidades de este estudio. No obstante, la revisión de la literatura pone de manifiesto que, al igual que ocurría con la antigüedad de la empresa, la relación entre edad e innovación no es totalmente clara. En este sentido, autores, como Khan y Manopichetwattana (1989) sostienen que ambas variables presentan una relación inversa, ya que serán los dirigentes más jóvenes los que estarán más dispuestos a incorporar las innovaciones.

Por el contrario, estudios como los de Lasch *et al.* (2007) o Álvarez y Otero (2007), señalan la existencia de una relación directa entre ambas variables, siendo los dirigentes de mayor edad los más propensos a incorporar las TIC en la empresa, debido a que la experiencia y los conocimientos adquiridos por el dirigente afectan de forma favorable a la actualización permanente de la empresa.

Por otro lado, otros autores como Verheul *et al.* (2002), Aubert *et al.* (2006) o González y Peña (2007) consideran que la edad del dirigente no afecta de manera lineal a la posibilidad de que las empresas adopten nuevas tecnologías, siendo los dirigentes de mediana edad los más idóneos para la adopción de las nuevas tecnologías.

- Formación académica

La formación académica de los gerentes de la empresa es también un factor determinante para poseer una mentalidad abierta y receptiva a las innovaciones, pudiendo esto favorecer la incorporación de las TIC en la empresa (26, 43).

- Conocimientos específicos en TIC

Aún más decisiva que la formación académica de los máximos responsables de la administración de la empresa, son sus conocimientos sobre TIC a la hora de adoptar estas en el funcionamiento organizativo y comercial de las empresas. A tal efecto, Nguyen y Barret (2006) inciden en la importancia de que los directivos posean un elevado nivel de conocimiento en TIC, tanto para la adopción de nuevas herramientas y versiones, como para obtener el máximo rendimiento de su uso.

El conocimiento de las TIC por parte de los máximos responsables de la administración de la empresa, además de ser determinante para la adopción directa de estas tecnologías, lo es también para obtener beneficios de las mismas, lo que volverá a revertir en una nueva inversión futura (39, 71). Pero también pueden ser un claro condicionante para el apoyo que estos ofrezcan a iniciativas innovadoras que pudieran surgir del resto de miembros de la empresa o ajenos a ella. Esta última afirmación es apoyada por Marchese y Jones (2011), al considerar que el nivel de conciencia tecnológica de la

dirección incentiva la adopción de TIC en la empresa, configurándose la conciencia tecnológica a partir del conocimiento de las bondades de las TIC.

Presiones para la introducción de las TIC en la empresa

Diversas investigaciones, como las de Iacovou *et al.* (1995) o Dembla *et al.* (2007), identifican a las presiones externas como una de las causas que explican el por qué las empresas deciden incorporar TIC en su organización. Según la literatura, las principales presiones externas proceden de los siguientes actores:

- Clientes y proveedores: Levy *et al.* (2005), Gebauer *et al.* (2005) o Alderete (2010).

- Administraciones públicas: Sadowski *et al.* (2002), Boer y During (2001) o Chong (2006).

- La competencia: Sadowski *et al.* (2002), Kim y Jee (2007) o Dembla *et al.* (2007).

Beneficios percibidos de la incorporación de las TIC

Los beneficios percibidos por las empresas es otro de los factores que han sido estudiados como influyentes en la configuración de una actitud innovadora y, por tanto, en la posibilidad de que las empresas adopten las TIC.

Los estudios realizados en este sentido llegan a una conclusión unánime: "Los beneficios percibidos influyen en la adopción de TIC por parte de las empresas" (21, 24, 26). De acuerdo con ello, es necesario que las empresas sientan seguridad plena de que se producirán dichos beneficios para que decidan tener una actitud proactiva al respecto.

Esta necesidad de percepción de beneficios se convierte en fundamental y es exigencia previa para adoptar la innovación en el caso de empresas de la

economía social, así como en el caso de las Pymes (44, 60). Se destacan estos últimos estudios, dado el perfil de las empresas de esta investigación, en la que cerca del 50 por 100 son empresas de economía social y casi un 92 por 100 son Pymes.

Planificación de las TIC

El estudio del proceso de planificación de las TIC se ha centrado, tradicionalmente, en su influencia sobre los resultados empresariales. Así, Sohal *et al.* (2006) sostienen la importancia de la planificación del uso de las TIC en las empresas para obtener de ellas el máximo rendimiento.

Por su parte, otros autores destacan la importancia de que el plan TIC esté alineado con la estrategia general de la empresa, adaptándose las empresas a estas tecnologías y estas, a su vez, a las características de la empresa y a sus líneas generales de actuación (32, 59).

METODOLOGÍA

Este estudio analiza las empresas españolas dedicadas a la comercialización del principal producto agroalimentario ecológico en España: el aceite de oliva ecológico. Esta población fue obtenida partiendo del directorio de empresas de producción ecológica, ofrecida por el Ministerio de Agricultura, Alimentación y Medio Ambiente del Gobierno de España (2007).

Posteriormente, se identificaron aquellas que disponían de sitio web propio a partir de los principales buscadores de Internet. De esta forma, son 127 las organizaciones que disponen de sitio web propio, sobre un total de 195 empresas productoras y comercializadoras de aceite de oliva ecológico en España.

Por tanto, son estas 127 empresas las que constituyen el universo objeto de este estudio.

A todas ellas se les realizó una encuesta telefónica con el objeto de obtener los datos necesarios para esta investigación, obteniendo respuesta de 99. Con este índice de respuesta se ha cubierto el 77,95 por 100 de la población estudiada (tabla 2). A título indicativo, si esta muestra hubiera sido aleatoria, el error máximo aproximado cometido hubiese sido del 4,74 por 100, con un nivel de confianza del 95,5 por 100.

En el presente trabajo se han aplicado exclusivamente técnicas e instrumentos de estadística descriptiva.

RESULTADOS Y DISCUSIÓN

A continuación se presentan los principales resultados obtenidos de este estudio.

Antigüedad de la empresa

Respecto de la antigüedad de las empresas objeto de estudio (tabla 3, pág. 241), puede observarse que

predominan las empresas de mediana edad (más del 60 por 100 de las empresas tienen entre 11 y 30 años de antigüedad). En cambio, es reducido el número de empresas con menos de 10 años (el 9,09 por 100), que pueden ser clasificadas como jóvenes.

Por otro lado, el número de empresas con más de 30 años es algo superior, representando cerca del 29 por 100 de estas entidades. Este elevado número de años de funcionamiento, si bien supone una experiencia añadida que ayuda a saber aprovechar las oportunidades del entorno (28), como el que brinda las TIC, también puede incorporar rigideces organizativas o comerciales que obstaculicen la adopción de estas tecnologías (58). Por tanto, se puede considerar la antigüedad como una variable cuya influencia para la adopción y aprovechamiento de las TIC es discutida en la literatura, sin existir unanimidad en su signo.

Tabla 2. Ficha técnica del estudio empírico.

Table 2. Technical data of the empirical study.

POBLACIÓN	
Unidades de muestreo	Sociedades españolas del sector del aceite de oliva ecológico con sitio web propio.
Población total	127 Sociedades.
Elementos de muestreo	Máximos responsables de la administración de las sociedades o aquellas personas a las que dirigía el anterior (miembros del consejo rector o responsables de la oficina).
Alcance	España.
Tiempo	1 de diciembre de 2012 al 5 de abril de 2013.
Encuestas válidas	99 sociedades.
Cubertura de repuesta	77,95 por 100.

Fuente: elaboración propia. / Source: self made.

Tabla 3. Antigüedad de las empresas del sector.

Table 3. Age of companies in the sector.

Años en funcionamiento	Nº de empresas	Porcentaje
0-10	9	9,09
11-20	42	42,43
21-30	17	17,17
31-40	5	5,05
41-50	9	9,09
Más de 50	15	15,15
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Tamaño de la empresa

En el caso de las empresas, la tabla 4 (pág. 242), muestra que la inmensa mayoría de organizaciones es de pequeña dimensión, atendiendo al criterio de volumen de facturación. De esta forma, según los criterios de clasificación establecidos por la Comisión Europea (European Commission, 2006), el 56,57 por 100 de las entidades son microempresas (con menos de 2 mill. de euros de facturación) y el 29,29 por 100 son pequeñas empresas (de entre 5 y 10 mill. de euros de facturación). Así, en este sector hay casi un 92 por 100 de Pymes, frente a tan solo algo más del 4 por 100 de grandes empresas (de más de 50 mill. de euros de facturación).

La pequeña dimensión de las empresas del sector puede explicar la reducida adopción de las TIC, debido a la mayor dificultad para acceder a recursos financieros, personal, etc. (6, 49). Así lo denotan autores como Tether (2005), al afirmar la menor propensión de las pequeñas empresas para adoptar innovaciones tecnológicas.

No obstante, algunos autores hablan de un cambio de tendencia, demostrando el aumento de propensión a innovar de las

Pymes (39). Por ello, se puede afirmar que en la actualidad existe un debate sobre la influencia del tamaño empresarial en la posibilidad de que las empresas se adapten a las innovaciones tecnológicas.

Recursos humanos

Como se aprecia en la tabla 5 (pág. 242), es mayoritario el número de empresas que tiene en su plantilla menos de 10 empleados a tiempo completo. Es decir, la inmensa mayoría de las empresas de la muestra pueden ser catalogadas como microempresas (un 83,33 por 100, de las cuales un 56,67 por 100 tienen menos de 5 empleados a tiempo completo). Este aspecto supone un hándicap negativo, dado que las tareas rutinarias no pueden ser delegadas en una parte de la plantilla, impidiendo la posibilidad de realizar cursos de reciclaje y adaptación a las nuevas tendencias del mercado, como el comercio electrónico (7, 37).

Por tanto, el reducido número de empleados en oficina puede ser una de las más importantes trabas a la formación y actualización de estos empleados.

Tabla 4. Volumen de facturación de las empresas.**Table 4.** Volume of turnover of the companies.

Tamaño	Nº de empresas	Porcentaje
Microempresa	56	56,57
Pequeña empresa	29	29,29
Empresa mediana	6	6,06
Empresa grande	4	4,04
Ns/Nc	4	4,04
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Tabla 5. Número de empleados a tiempo completo.**Table 5.** Number of full-time employees.

Nº de empleados	Nº de empresas	Porcentaje
Menos de 5	56	56,67
5-9	26	26,26
10-15	6	6,06
16-20	3	3,03
21-25	2	2,02
Más de 25	6	6,06
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Máximo responsable de la administración

El análisis del origen de las iniciativas innovadoras en la empresa, tabla 6 (pág. 243), pone de manifiesto el importante peso que tiene la figura del máximo responsable de la administración. Así, en un 14,14 por 100 de las ocasiones proceden del presidente o consejo rector (en el caso de las sociedades cooperativas), un 31,32 por 100 surgen del gerente de la empresa y un 4,04 por 100 del propietario.

En términos globales, en un 52,60 por 100 de empresas, las iniciativas surgen de manera exclusiva de los órganos decisores o directivos de la empresa, porcentaje al que se debe añadir el 13,13 por 100 de

empresas en las que la iniciativa procede de manera conjunta de empleados y gerente.

Este papel protagonista que el máximo responsable de la organización tiene en la toma de decisiones relacionadas con la adopción de las TIC justifica analizar las características que definen su perfil.

Edad

Tal y como se desprende de la tabla 7 (pág. 243), en las empresas estudiadas no proliferan los dirigentes demasiado jóvenes (con menos de 30 años), ni tampoco los que superan la edad de 60 años (8,08 por 100 de las empresas analizadas).

Tabla 6. Iniciativa para introducir o incrementar las TIC en la empresa.

Table 6. Initiative to introduce or increase ICT in the company.

Respuesta	Nº de empresas	Porcentaje
Presidente/C. Rector	14	14,14
Gerente	31	31,32
Empleados con conocimientos TIC	28	28,28
Gerente y empleados	13	13,13
Presidente y empleados	1	1,01
Gerente y presidente/C. Rector	1	1,01
Propietario	4	4,04
Jefe de administración	1	1,01
Director de <i>marketing</i>	1	1,01
Grupo empresarial	1	1,01
Nadie/obligación	2	2,02
Ns/Nc	2	2,02
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Tabla 7. Edad del máximo responsable de la administración.

Table 7. Maximum responsible of the administration's age.

Edad	Nº de empresas	Porcentaje
Menos de 30	1	1,01
30-40	28	28,28
41-50	35	35,36
51-60	26	26,26
Más de 60	8	8,08
Ns/Nc	1	1,01
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Por tanto, el perfil del dirigente de las empresas oleícolas de producción ecológica en España es de mediana edad, ya que en la mayoría de ellas (en el 63,64 por 100), el dirigente tiene una edad comprendida entre los 30 y 50 años.

Según la literatura más reciente, esta mediana edad de los gerentes de las empresas puede ser positivo para la adopción de innovaciones tecnológicas (3, 26, 74).

Formación académica

Como se observa en la tabla 8 (pág. 244), existe un alto porcentaje de dirigentes de las empresas estudiadas que tienen formación universitaria: los diplomados y licenciados suponen un 64 por 100 en este trabajo. Esta elevada formación académica favorece la existencia de una actitud receptiva hacia la adopción de elementos TIC en la empresa (26, 43).

Tabla 8. Nivel académico del máximo responsable de la administración.**Table 8.** Academic level of the head of administration.

Nivel académico	Nº de empresas	Porcentaje
Sin estudios	1	1,01
Estudios primarios	11	11,11
Bachiller/FP II	21	21,21
Diplomado	27	27,27
Licenciado	36	36,37
Ns/Nc	3	3,03
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Formación en TIC del máximo responsable de la empresa

Con relación a la formación en TIC de los dirigentes de las empresas oleícolas estudiadas, la información recogida en la tabla 9, muestra que no puede considerarse elevada: tan solo un 14,14 por 100 de ellos tienen un nivel avanzado de conocimientos en TIC. Esta característica obstaculiza la adopción de elementos TIC en las empresas del sector y su óptimo aprovechamiento en su actividad comercial (39, 56).

Presiones para la adopción de las TIC

Del análisis de las presiones recibidas por las empresas del sector del aceite de oliva ecológico estudiados en esta investigación (tabla 10, pág. 245), llama la atención que un elevado porcentaje de las mismas manifiesten no haber tenido ninguna presión que les obligase a incorporar las TIC (un 79,80 por 100 de empresas).

De las empresas que sí afirman haber tenido presiones, destaca sobre los demás actores, los clientes (15 empresas).

Las escasas presiones procedentes del exterior para adoptar las TIC es otro elemento que desanima su introducción en la empresa (19).

Tabla 9. Formación en TIC del máximo responsable de la administración.**Table 9.** ICT training of the head of the administration.

Formación en TIC	Nº de empresas	Porcentaje
Sin conocimientos	10	10,10
Nivel de usuario	32	32,32
Nivel intermedio	40	40,41
Nivel avanzado	14	14,14
Ns/Nc	3	3,03
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Tabla 10. Procedencia de las presiones para incorporar las TIC en la empresa.

Table 10. Origin of pressures to incorporate ICT in the company.

Respuesta	Nº de empresas	Porcentaje
Empresas clientes	1	1,01
Consumidores directos	1	1,01
Administraciones públicas (AAPP)	1	1,01
Proveedores y empresas clientes	1	1,01
Empresas clientes y AAPP	2	2,02
Consumidores directos y empresas clientes	7	7,07
Competencia	1	1,01
Grupo empresarial	1	1,01
Proveedores y consumidores directos	1	1,01
Trabajadores y socios	1	1,01
De todos	2	2,02
No he tenido presiones	79	79,80
Ns/Nc	1	1,01
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Beneficios percibidos de la introducción de las TIC

Al objeto de averiguar si las TIC eran valoradas positivamente por las empresas como herramientas para incrementar sus ventas, se preguntó sobre la importancia que para la venta en Internet tiene la imagen de su producto en este medio. Los resultados obtenidos (tabla 11, pág. 246) no dejan lugar a equívoco: las empresas oleícolas de producción ecológica en España consideran fundamental la imagen del producto en Internet para potenciar su venta.

Del estudio de esta variable se observa una clara unanimidad en la literatura especializada, al señalar que los beneficios percibidos son un factor determinante para que las empresas realicen innovaciones e implanten tecnológicas (21, 24, 26).

A continuación, se pregunta a las empresas si el empleo de las TIC contribuye a mejorar su imagen y la de sus productos. El objetivo es comprobar si, desde el punto de vista de las empresas estudiadas, el

manejo de las herramientas TIC (tiendas electrónicas en el sitio web, mercados electrónicos, redes sociales, etc.) ayuda a mejorar la percepción que sus actuales o potenciales clientes tienen de la empresa y de sus productos. Según los datos de la tabla 12 (pág. 246), un elevado porcentaje de empresas (un 79,80 por 100) considera que el uso de TIC contribuye a mejorar su imagen y la de sus productos: un 37,38 por 100 de ellas están bastante de acuerdo con esta idea, siendo el 42,42 por 100, las que están totalmente de acuerdo con ella.

A la vista de estos datos se puede afirmar que las empresas de esta investigación tienen una imagen positiva de las TIC, al mismo tiempo que perciben que la adopción y uso de estas ofrecerán beneficios a su empresa. Por lo tanto, esta buena imagen de las TIC puede influir positivamente en la existencia de una actitud proactiva hacia su adopción en el futuro (21, 44).

Tabla 11. Valoración de la afirmación: "la imagen del producto en la red es un componente esencial para su venta".

Table 11. Valuation of the claim: "the image of the product in the network is an essential component for its sale".

Valoración	Nº de empresas	Porcentaje
En total desacuerdo	2	2,02
En desacuerdo	6	6,06
Indiferente	11	11,11
De acuerdo	34	34,34
Totalmente de acuerdo	45	45,46
Ns/Nc	1	1,01
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Tabla 12. Valoración de la afirmación: "el empleo de TIC contribuye a mejorar la imagen de la empresa y de sus productos".

Table 12. Assessment of the statement: "the use of ICT contributes to improving the image of the company and its products".

Valoración	Nº de empresas	Porcentaje
En total desacuerdo	0	0,00
En desacuerdo	2	2,02
Indiferente	17	17,17
De acuerdo	37	37,38
Totalmente de acuerdo	42	42,42
Ns/Nc	1	1,01
TOTAL	99	100,00

Base: empresas con sitio web propio. / Fuente: elaboración propia.

Base: companies with their own website. / Source: self made.

Planificación de las TIC

Como se observa en la tabla 13 (pág. 247), la inmensa mayoría de las empresas objeto de la investigación (un 89,90 por 100) no tenían en el año 2012 un plan de TIC.

Esta falta de planificación en las empresas puede justificarse fundamentalmente en sus características estructurales, principalmente en la reducida dimensión de estas organizaciones.

Así, investigaciones como las de Kowtha y Choon (2001) sostienen que la planificación en TIC está relacionada con el tamaño de las empresas.

Cabe considerar que la incorporación de las herramientas TIC no puede ser considerada como un paso más que la empresa da en su día a día; no es una estrategia operativa, sino que debe ser considerada como una decisión estratégica, es decir, a largo plazo, que requiere un riguroso control en su planificación e implementación (31).

Tabla 13. ¿Existió en el año 2012 un plan de tecnologías de la información y comunicación en la empresa?

Table 13. Was there in 2012 a plan for information and communication technologies in the company?

Respuesta	Nº de empresas	Porcentaje
Sí	10	10,10
No	89	89,90
TOTAL	99	100,00

Base: empresas con sitio web propio.

Fuente: elaboración propia.

Base: companies with their own website.

Source: self made.

CONCLUSIONES

El mercado de productos agroalimentarios ecológicos en España se enfrenta a un exceso de oferta provocado por la existencia de una de las mayores ofertas de estos productos a nivel mundial y, al mismo tiempo, de una demanda interna prácticamente inexistente. En este contexto, las TIC tienen el potencial de dar respuesta a estos problemas comerciales, por los múltiples beneficios y fortalezas que se derivan de su aplicación para fines empresariales.

De acuerdo con ello, y tomando como objeto de estudio uno de los principales sectores agroalimentarios ecológicos en España, esto es, el del aceite de oliva, el presente trabajo ha tratado de analizar si aquellas características que la literatura señala como relevantes para explicar la adopción y uso eficiente de las TIC en la empresa, están efectivamente presentes entre las empresas de dicho sector.

Los resultados obtenidos muestran que el perfil de las empresas analizadas tiene importantes debilidades estructurales, como: escaso número de trabajadores disponibles, nivel de formación de los directivos en TIC medio-bajo,

posibles problemas de comunicación con su entorno, puesto que la dirección no percibe presiones para utilizar/implantar TIC, ni para realizar una planificación de inversiones en estas tecnologías. Pese a ello, estas empresas cuentan con otras características que sí pueden favorecer la adopción de nuevas herramientas TIC o actualizaciones de estas, como son: la mediana edad de sus directivos, el nivel académico medio-alto de sus directivos y la percepción de los beneficios que puede reportar para las empresas la adopción de estas tecnologías.

Por lo tanto, se puede decir que el perfil organizativo de las empresas de este sector no es el idóneo para esperar de ellas el máximo aprovechamiento de las posibilidades que le ofrece el entorno tecnológico actual, aunque existen factores que pueden llamar al optimismo.

Llegados a este punto es preciso señalar las principales limitaciones presentes en este estudio. Por un lado, cabe mencionar que esta investigación se ha dirigido especialmente a las entidades del sector oleícola ecológico, aunque se estima que estas aportaciones pueden ser extrapolables a una gran parte del sector agroalimentario, que presenta, en términos generales, un problema similar de fondo en materia de comercialización. Además, este estudio se centra en las entidades con sitio web propio. Aunque este aspecto también limita la generalización de las conclusiones, se considera que las deficiencias organizativas serán similares o, en su caso, peores para el resto de empresas del sector, dado que las entidades estudiadas, al menos, cuentan actualmente con un mínimo contacto y adaptación a las TIC. Por otra parte, también se señala como limitación que este estudio se ha enfocado al ámbito nacional. En este sentido, pese a que

España ocupa una posición privilegiada en la producción de este cultivo, puede ser interesante contrastar su situación con la que acontece en otros países productores.

Para finalizar, este estudio contribuye a aportar nuevos conocimientos a una cuestión poco tratada en la literatura, como es la idoneidad de la situación estructural y organizacional del sector agroalimentario ecológico para la adopción y utilización de las TIC. En él se destacan una serie de deficiencias que las empresas

del sector deben corregir para su mejora y avance comercial. Por tanto, este estudio puede servir al sector del aceite de oliva ecológico como indicador de los aspectos clave que deben ser tenidos en cuenta para la renovación del sector y la obtención de una mejor orientación al actual mercado.

Futuras investigaciones deberían centrar su atención en el avance de estos aspectos, así como en comprobar si dichos avances tienen una repercusión directa en los resultados comerciales de las empresas.

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Percepción de los residentes sobre el desarrollo de la micro región Tierra de Palmares desde la perspectiva del turismo rural integrado

Perception of residents on the development of the micer region Tierra of Palmares from the integrated rural tourism perspective

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RESUMEN

El objetivo de este trabajo es analizar las percepciones de los residentes sobre las variables implicadas en el desarrollo del Turismo Rural Integrado (TRI) así como sus efectos sobre dicho desarrollo. Para ello, se analiza el caso concreto de los residentes de Tierra de Palmares, micro región implicada en un proyecto de desarrollo turístico. Los residentes constituyen uno de los principales actores para que este tipo de turismo pueda desarrollarse de manera exitosa. El análisis descriptivo realizado así como el análisis de Componentes Principales, muestra que los residentes perciben la existencia de las variables clave para alcanzar el tipo de turismo deseado. Así, se comprueba que se percibe la existencia de colaboración entre los agentes implicados y el carácter arraigado, endógeno y complementario de las actividades turísticas desarrolladas, las cuales permiten el empoderamiento de los residentes. Además, los efectos positivos del turismo también se están alcanzando, ya que se percibe la existencia de sostenibilidad económica, sociocultural y medioambiental. Los resultados de las regresiones realizadas muestran que los efectos de todas estas variables sobre el desarrollo del TRI son positivos aunque deben ser analizados con mayor profundidad. Así, se detecta el efecto positivo sobre el TRI de la existencia de redes informativas, del empoderamiento de los residentes, del desarrollo del turismo como complementario a las actividades tradicionales y del uso compartido de recursos con los turistas. Sin embargo, la inclusión de las sostenibilidades en la regresión diluye el efecto ejercido por la complementariedad y el uso compartido. Estos resultados ponen de manifiesto la necesidad de plantear relaciones indirectas entre todas las variables potencialmente determinantes del TRI, las cuales mejorarían la comprensión de su desarrollo.

Palabras clave

turismo rural integrado • residentes • micro región • redes • arraigo • endogeneidad • complementariedad • empoderamiento • sostenibilidad económica • sostenibilidad sociocultural • sostenibilidad medioambiental

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ABSTRACT

The objective of this work is to analyze the perceptions of residents regarding the variables involved in developing Integrated Rural Tourism (IRT), as well as analyze how those variables affect said development. Therefore, we have analyzed the specific case of the residents of Tierra de Palmares, which is an area involved in a tourism development project. The residents constitute one of the main actors involved in the successful development of this type of tourism. The descriptive analysis performed as well as the Principal Components analysis shows the residents perceive the existence of key variables for reaching the desired type of tourism. Thus, it is verified that there is cooperation between the agents involved and the embedded, endogenous and complementary nature of the tourism activities that are developed, which activities allow empowering the residents. Moreover, it seems that positive effects are being generated by tourism, given that the existence of economic, sociocultural and environmental sustainability is perceived. The results of the regressions performed show that the effects of all these variables on the development of IRT are positive, although they should be analysed more in depth. Thus, the positive effect on IRT of the existence of information networks, the empowerment of residents, the development of tourism as complementary to traditional activities and the sharing of resources with tourists is detected. However, the inclusion of sustainability in the regression dilutes the effect exerted by complementarity and sharing. These results demonstrate the need to establish indirect relationships between all potentially determinant variables of TRI, which would improve the understanding of their development.

Keywords

integrated rural tourism • residents • micro-region • networks • embeddedness • endogeneity • complementarity • empowerment • economic sustainability • socio-cultural sustainability • environmental sustainability

INTRODUCCIÓN

El turismo es uno de los principales sectores de la economía mundial, que implica importantes transferencias de riqueza, y favorece el desarrollo y el crecimiento económico de las zonas de destino. En 2014, la contribución total del turismo a la economía global fue de 7,6 billones de dólares, lo que equivale al 9,8% del PIB de toda la economía de ese año y supone un crecimiento del 3,6% respecto del año anterior. Además, la contribución total del turismo al empleo también creció un 2,3% en ese año. Estos datos demuestran

que, a pesar de la crisis, el sector turístico constituye un motor clave para el continuo crecimiento global y para la creación de empleo, manteniéndose estas expectativas para los próximos años (86).

Este papel del turismo como un importante agente de crecimiento económico ha sido ampliamente adoptado por organismos oficiales (37, 58). Así, conscientes de su importancia, muchos gobiernos y organizaciones consideran al turismo como una actividad económica muy prometedora que puede ser una

herramienta clave para el desarrollo de determinadas regiones (50, 61). Pero, a pesar de estos datos favorables, se debe tener en cuenta que la magnitud y el crecimiento del turismo ejerce una gran presión tanto hacia el medioambiente como hacia los recursos socioculturales (66). Así, el turismo ejerce una serie de importantes efectos económicos, sociales, culturales y ambientales que es imprescindible considerar de cara a gestionarlos con eficiencia (19, 44, 45).

Las características del entorno rural, entre las que se encuentran la reestructuración del sector agrícola y la emigración de los jóvenes a las ciudades (10), hacen que el turismo sea contemplado como una posible herramienta para la reactivación socioeconómica de estas regiones, ya que permite enfrentarse al estancamiento económico y prevenir la emigración (25, 50). Determinadas zonas rurales son percibidas como poseedoras de un elevado potencial para desarrollar el turismo, dada la existencia de recursos como los paisajes naturales, las culturas y tradiciones rurales, la posibilidad de hacer actividades al aire libre o la gastronomía (54). Las industrias tradicionales existentes en estas zonas pueden ver complementadas sus actividades a través del turismo, pudiendo formar un atractivo producto turístico que responda a las demandas de nuevas experiencias por parte de los turistas. De esta manera, el turismo puede constituir una alternativa para la diversificación y la reconversión de las áreas rurales (23, 80; 86).

Sin embargo, el desarrollo turístico no solo genera beneficios que contribuyen al desarrollo de las regiones rurales. También puede generar impactos negativos en las estructuras de la zona, en su entorno cultural, en sus estructuras sociales y patrones de consumo, en el empleo y en

el medioambiente, siendo la saturación el principal riesgo en el que se incurre.

Como recoge Sánchez (2011), esta saturación contribuye a la pérdida de la identidad local (Banducci & Barreto, 2001), del legado cultural (Barreto, 2000), y del paisaje cultural (Meneses, 2000). Debe tenerse en cuenta que en el turismo rural participan una gran diversidad de actores, instalaciones y recursos, entre los que existen múltiples relaciones e interacciones. Si estos elementos no son considerados y gestionados correctamente pueden obstaculizar el camino hacia un desarrollo sostenible. La sostenibilidad se ha convertido en una de las variables clave para lograr la competitividad de un destino (21, 85). Sin embargo, conseguir un turismo sostenible no es fácil, ya que la naturaleza imprecisa de este concepto hace que sea de difícil aplicación.

En general, se habla de un turismo sostenible cuando se aplican los principios de desarrollo sostenible en sus dimensiones económica, medioambiental y social. Por tanto, para gestionar esta compleja realidad, se hace necesario el uso de un marco de análisis integral y un modelo de desarrollo turístico holístico.

Como respuesta a esta necesidad surge el concepto de Turismo Rural Integrado (TRI). El TRI puede comprenderse como el turismo que está explícitamente relacionado con las estructuras económicas, sociales, culturales, naturales y humanas del entorno rural en el que se desarrolla (25).

La necesaria implicación de los residentes de la zona para que este tipo de turismo sea gestionado con éxito (36, 58), hace que el conocimiento de las opiniones de los residentes sobre su desarrollo resulte clave (5, 30, 82). Por ejemplo, las actitudes negativas de los residentes hacia el turismo pueden constituir una barrera

para el desarrollo de un destino turístico (20, 31), ya que el éxito del destino depende de los atractivos locales y de la hospitalidad de los residentes (29). Tal y como establecen Brida, Osti y Barquet (2010), los residentes constituyen los principales actores a quienes afecta el desarrollo turístico. Solo se justifica llevar a cabo un desarrollo turístico si este mejora la calidad de vida de los residentes o si, al menos, no la deteriora (4, 22, 39, 75, 77). En definitiva, son los residentes los que deciden qué impactos del turismo son aceptables y cuáles no lo son. Por tanto, supervisar periódicamente las opiniones de los residentes resulta necesario a la hora de desarrollar un proyecto turístico, ya que permite desarrollar políticas que minimicen los efectos negativos del turismo y maximizar los positivos (79, 83). El impacto percibido del turismo es uno de los principales predictores del apoyo de los residentes al desarrollo del turismo sostenible en su comunidad (43).

El objetivo principal de esta investigación pretende conocer si los residentes de una región inmersa en las fases iniciales de desarrollo de un Turismo Rural Integrado perciben la existencia de las principales variables implicadas en el mismo. En caso afirmativo, se pretende establecer las relaciones percibidas que existen entre estas variables y el TRI, de tal manera que se pueda anticipar las intenciones de los residentes de participar en el desarrollo exitoso del proyecto.

Variables implicadas en el Turismo Rural Integrado

La noción de Turismo Rural Integrado es empleada por Saxena y Ilbery (2007) como una herramienta para el desarrollo de determinadas regiones rurales rezagadas. Uno de sus elementos clave es la integración de las variables en las que se sustenta, fundamentalmente económicas y sociales,

siendo su objetivo la consecución de un turismo sostenible. En términos prácticos, el TRI se refiere al turismo que tiene claras conexiones con los recursos, productos y habitantes locales (25, 38, 57, 71). Su principal objetivo es promover la sostenibilidad medioambiental, económica y socio-cultural del sistema rural, ya que el poder de decisión y de actuación radica en los habitantes de la zona (14).

La noción de integración muestra la tendencia a la creación de relaciones entre los diversos actores y recursos a través de redes de cooperación y de colaboración (69), de tal manera que se consiga que la maximización del valor añadido generado se quede en la zona (52).

La revisión de la literatura especializada muestra que la concepción del TRI se hace visible a través de una serie de variables clave: redes, arraigo, endogeneidad, complementariedad, empoderamiento y sostenibilidad, (14, 35, 38, 71).

En el TRI el concepto de redes se aborda desde su perspectiva más amplia, refiriéndose a las relaciones sociales dinámicas a través de las cuales los agentes pretenden alcanzar objetivos (70). Por tanto, pueden entenderse como los lazos relacionales que conectan a un conjunto de actores, grupos e instituciones, que son capaces de unir personas y organizaciones de diferentes entornos e intereses (60).

En las regiones rurales es importante proveer un producto turístico innovador que atraiga y retenga a los turistas en el destino durante varios días. Sin embargo, las organizaciones existentes suelen ser pequeñas organizaciones familiares, con escaso capital económico y personal, por lo que sus capacidades son limitadas.

En este contexto, las asociaciones entre los distintos agentes encargados de desarrollar y de promocionar el turismo en la zona resultan vitales para el desarrollo del TRI (14, 16, 38, 71). El concepto de

redes engloba a todos los vínculos formales e informales de cooperación, colaboración o integración entre empresas, instituciones y/o individuos que tienen como objetivo desarrollar el TRI en la zona.

Para mantener la conexión con los recursos de la zona, es necesario que el turismo enfatice el contexto territorial donde se van a llevar a cabo las actividades turísticas, reafirmando la construcción de la identidad local a través del arraigo. Esta característica describe una actividad turística que es parte de la vida social y recreacional local, y donde los productos ofertados tienden a tener marcas, etiquetas o acreditaciones locales. Por tanto, el arraigo sugiere que los recursos y actividades turísticas están directamente relacionados con el lugar donde se llevan a cabo, y que esas relaciones se forman en un determinado contexto sociocultural proporcionando valores compartidos, símbolos y tradiciones (32, 59). Por tanto, existe arraigo cuando las actividades turísticas son parte de la vida social, cultural y recreacional de la comunidad y/o los productos turísticos reflejan el entorno local (57).

Además del arraigo de los recursos, el TRI pretende conseguir un desarrollo endógeno del turismo, lo que implica la existencia de una estructura capaz de retener el máximo de beneficios en la localidad, usando y agregando valor a los recursos locales y centrándose en los requerimientos, capacidades y valores de los actores locales (65). Por tanto, se pretende que los recursos donde se base el desarrollo del turismo de la zona, cultural, histórico y/o medioambiental, sean de propiedad local o estén gestionados por miembros de la comunidad.

Este tipo de turismo no pretende sustituir las actividades agrícolas de la zona por actividades turísticas, si no que pretende una complementariedad entre

ellas (38). De esta manera, el entorno rural sigue manteniendo su actividad principal tradicional, obteniendo ingresos complementarios derivados de las actividades turísticas.

El turismo del vino es un buen ejemplo de esta complementariedad (12). Además, la complementariedad también implica que turistas y residentes usen de manera conjunta los recursos existentes, viéndose estos mejorados por la existencia de la actividad turística (16).

Los resultados alcanzados a través de este tipo de desarrollo turístico deben ser sostenibles, esto es, que equilibren tanto las aspiraciones sociales, económicas y medioambientales de las comunidades como los costes y beneficios de los actores clave, no deteriorando la calidad de los recursos existentes (71).

El aprovechamiento sostenible de los recursos y actividades tiende a la viabilidad económica y a la conservación sociocultural de los mismos. Por lo tanto, el desafío se encuentra en la continua búsqueda y en el fomento de formas benignas de turismo que se adecuen de la mejor manera posible al criterio de desarrollo económico y social del destino (73). Para ello, el grado de desarrollo del turismo debe ajustarse a las infraestructuras existentes, evitando el deterioro de los recursos de la zona.

En todo este proceso de desarrollo resulta clave la implicación de los residentes y su participación. Para ello, es necesario hacerles comprender los objetivos que la comunidad se plantea alcanzar a través del desarrollo turístico así como las ventajas que se derivan. Además, deben tomar parte del proceso, manifestando sus opiniones y haciéndoles ver que estas son tenidas en cuenta. Este empoderamiento de los residentes a través de la administración y control de

los recursos de los que depende, puede generar beneficios para el desarrollo del turismo y una alta motivación para conservar y hacer un uso sostenible de los recursos (40).

Por tanto, se establece que el desarrollo del TRI está ligado a las estructuras económicas, socioculturales y naturales del entorno donde se desarrolla, se construye a través de redes sociales endógenas y arraigadas, es complementario con las actividades tradicionales, empodera a las comunidades locales, siendo su objetivo promover la sostenibilidad económica, sociocultural y medioambiental del amplio sistema rural.

Importancia de las percepciones de los residentes

Para alcanzar el éxito en el desarrollo turístico, es fundamental conseguir un equilibrio en las relaciones que se establecen entre los turistas, los residentes, los lugares donde se encuentran, y las organizaciones encargadas de proporcionar los servicios turísticos (83). Lógicamente, los distintos grupos de la comunidad que participan en el desarrollo turístico pueden tener diferentes percepciones sobre dicho desarrollo (3), y todas ellas deben ser objeto de estudio.

La literatura académica establece que los residentes constituyen el principal actor del proceso de desarrollo turístico ya que se ven directamente afectados por dicho proceso (5, 28, 53). Así, las actitudes que muestren los residentes ante el desarrollo turístico constituyen uno de los principales predictores de dicho desarrollo, de tal manera que si las actitudes son positivas, los residentes favorecerán su desarrollo (18, 43, 53, 55, 76). Por tanto, estas actitudes afectan a la puesta en práctica de los procesos de planificación de los destinos (41), al desempeñar un impor-

tante papel estratégico en el desarrollo del turismo. Además de sus efectos sobre la satisfacción de los turistas (26), se debe destacar la relevancia que la oposición activa de los residentes al turismo tiene sobre el resto de actores implicados, fundamentalmente, gobiernos locales, empresas turísticas e intermediarios (17, 24). De esta manera, los residentes se pueden convertir en el mejor aliado o en el peor enemigo del desarrollo de un destino.

Todo esto es especialmente relevante en el caso del TRI, en el cual resultan claves las percepciones de los residentes para lograr su éxito (1, 5, 29, 30). Dado que los objetivos del TRI se centran en conseguir un turismo sostenible, el apoyo directo o indirecto que presten los residentes constituyen uno de los elementos clave para su desarrollo (11, 34, 36). La sostenibilidad requiere del equilibrio entre los efectos negativos y positivos que causa el turismo en sus tres principales dimensiones: social, económica y medioambiental (2, 64, 67, 82). Efectos que han sido ampliamente estudiados en la literatura académica, tal y como muestra la revisión realizada por Sharpley (2014). El desarrollo de un turismo rural y sostenible solo puede verse justificado si sirve para mejorar la calidad de vida de los residentes o, al menos, de no deteriorarla (15, 22, 39). Por tanto, es necesario armonizar los intereses de los residentes y los del resto de actores implicados en su planificación, lo cual se consigue a través del TRI. Como se ha indicado, entre sus elementos clave se encuentra el empoderamiento de los residentes, asegurando su participación en los procesos de toma de decisiones, así como revertir los ingresos derivados del turismo en la comunidad, convirtiendo a los recursos existentes en autosuficientes (46, 78).

METODOLOGÍA

Para alcanzar los objetivos del estudio, se procede a analizar la información proporcionada por los residentes de las localidades de los departamentos de Colón y San Salvador, situados en la provincia de Entre Ríos (Argentina). Estas zonas se caracterizan por la riqueza y variedad de sus recursos naturales (playas de río, aguas termales, parque nacional "El Palmar") y culturales (colonias agrícolas fundadas por inmigrantes europeos).

Hasta 1984 la provincia de Entre Ríos no fue incorporada a los planes de desarrollo del turismo que intentó implementar el Ministerio de Economía de Argentina. En 2005 el gobierno nacional lanzó el Plan Federal Estratégico de Turismo Sustentable 2016 (PFETS) que, para el caso de Entre Ríos, reconoce al Corredor Turístico del Río Uruguay como prioritario (49). Pero, dado que los corredores abarcaban un amplio territorio que impedía plasmar la esencia de sus localidades, el gobierno provincial puso en marcha un plan centrado en el turismo como alternativa de desarrollo de las economías regionales (84). En él se crearon distintas micro regiones turísticas articuladas por las principales localidades, rutas y accesos. Las micro regiones incluyen espacios rurales y urbanos que se complementan entre sí ya que comparten rasgos como la historia, la cultura, el paisaje o la naturaleza. Entre estas micro regiones se encuentra, desde 2007, Tierra de Palmares, integrada por los departamentos de Colón y San Salvador cuyas autoridades empezaron a ejecutar el desarrollo de un destino turístico conjunto. Su objetivo es el desarrollo turístico de la micro región teniendo como base el desarrollo sostenible y armónico de la actividad turística y como principios rectores el respeto de las aspiraciones

comunes de los residentes y la satisfacción de los visitantes (27).

La diversa oferta turística de la micro región (extensas áreas naturales, playas, aguas termales, sitios históricos-culturales, productivos, gastronómicos, recreativos y el Parque Nacional El Palmar) se agrupa configurando distintos itinerarios. Se pretende que el turista recorra la mayor parte de los atractivos que ofrece la micro región, cuya imagen se centra en el turismo de naturaleza dentro de Colón (13).

Para obtener la información necesaria, se realizó una encuesta personal a residentes de la zona durante los meses de enero y febrero de 2012. Debido a las restricciones temporales y especialmente presupuestarias para la realización del estudio, fue necesario realizar un muestreo de conveniencia. De esta manera, se obtuvieron 377 cuestionarios válidos para los objetivos del estudio, es decir, que proporcionan información de todas las variables implicadas en el TRI. Además, se obtuvo información sobre las características personales de los entrevistados.

Con la información obtenida se pretende determinar si los residentes perciben la existencia de las variables implicadas en el desarrollo del TRI así como el efecto de dichas variables sobre el desarrollo del proyecto. Para ello, en primer lugar se procede a realizar un análisis descriptivo de los ítems bajo estudio. Tras ello, se determina si existe una estructura subyacente en dichos ítems que permita establecer la existencia de las variables implicadas en el TRI. Para ello, se realiza un análisis de componentes principales con rotación *varimax*.

La comprobación de la existencia de estas variables permite realizar un análisis de regresión y determinar así sus potenciales efectos sobre el TRI.

Medición de las variables

Las variables empleadas en el estudio no son directamente observables, por lo que se deben operacionalizar para poderlas medir. Esta operacionalización consiste en disminuir el nivel de abstracción de la variable no observable a través de la delimitación de sus dimensiones y elementos (68).

El cuestionario empleado se inicia con una breve presentación del estudio. Tras ello, se presentan 3 bloques de ítems: i) indicadores de las variables de redes, arraigo, endogeneidad, complementariedad y empoderamiento; ii) indicadores de sostenibilidad económica, sociocultural, medioambiental y de Turismo Rural Integrado; y, finalmente, iii) características sociodemográficas de los individuos encuestados.

La mayoría de los ítems empleados fueron medidos a través de escalas tipo

Likert de once puntos, desde 0 "Totalmente en Desacuerdo" hasta 10 "Totalmente de Acuerdo", excepto las variables sociodemográficas.

Descripción de la muestra

Las características de la muestra se recogen en la tabla 1. La edad media de los encuestados se sitúa en los 36,2 años, siendo el valor más frecuente 30 años. Atendiendo a los rangos de edad, se observa que el 58,4% de la muestra tiene menos de 35 años.

Cabe destacar que la muestra está integrada mayoritariamente por mujeres (64,51%). Estas características de edad y sexo se corresponden con la distribución de la población existente en la zona (<http://www.entrerios.gov.ar/>).

Tabla 1. Características de la Muestra.

Table 1. Characteristics of the sample.

Sexo		Nivel de Estudios	
Hombre	35,5%	Sin Estudios	0,6%
Mujer	64,5%	Primario	9,1%
Edad		Secundarios	43%
Menos de 25	20,3%	Terciarios	28,4%
De 26 a 35	38,1%	Universitarios	17,1%
De 36 a 45	18,3%	Posgrado	1,9%
De 46 a 55	13,6%	Zona Residencia	
Más de 55	9,7%	Colón	42,7%
Estado Civil		Villa Elisa	18,9%
Soltero	37,1%	San José	16,7%
Casado	36,8%	San Salvador	8,8%
En convivencia	18,1%	Ubajay	3,3%
Divorciado	5,7%	Centros rurales	9,6%
Viudo	2,3%	Años de Residencia en la Zona	
Vinculación con el Turismo		Menos de 6	6,5%
Sí	35,6%	De 6 a 15	10,2%
No	63,3%	De 16 a 25	26,6%
		Más de 25	56,7%

Fuente: Elaboración propia / Source: own elaboration

La mayoría de los individuos (54,9%) están casados o viven en pareja. En cuanto al nivel de estudios, la mayor parte de la muestra (43%) ha finalizado los estudios secundarios, un 28,4% cuenta con estudios terciarios y un 17,1% con estudios universitarios. Además, el 42,7% de la muestra residen en Colón, la principal ciudad de la zona, el 18,7% en Villa Elisa, el 16,9% en San José, el 8,8% en San Salvador, y un 3,3% en la ciudad más pequeña, Ubajay. Además, un 9,6% de los encuestados residen en poblaciones con menos de mil habitantes (Liebig, San Anselmo, Hugues, Hocker y El Carmen).

En cuanto a los años de residencia en la zona, se observa que más de la mitad de la muestra (56,7%) son residentes antiguos, ya que viven en la zona desde hace más de 25 años.

Finalmente, destacar que el 35,9% de los encuestados desarrolla o ha desarrollado alguna actividad económica relacionada con el turismo (alquiler de inmuebles a turistas, empleo en establecimientos hoteleros, gastronómicos o complejos turísticos, en tiendas de productos regionales así como guía turístico).

RESULTADOS

Para alcanzar los objetivos planteados se procede a analizar la información obtenida. En primer lugar, se determinan las principales medidas de tendencia central (media y moda) y de variabilidad (desviación típica) de los ítems analizados. Además, con el objeto de analizar si estos valores son estadísticamente diferentes al valor central de la escala (el 5), se realiza el correspondiente contraste de medias, prueba T.

Los resultados obtenidos se muestran en la tabla 2 (pág. 262-263).

Los indicadores relacionados con la existencia de redes empleados son 8 (P1 a P8).

Los valores medios obtenidos son estadísticamente iguales o mayores que el punto medio de la escala empleada, lo que indica que los residentes perciben cierta colaboración entre los agentes implicados en el desarrollo turístico. Así, el mayor valor medio obtenido (6,60) se obtiene en "Existe colaboración entre las empresas e instituciones de la zona para la organización de eventos, festivales, etc.", (P4).

La relación entre los recursos y actividades turísticas desarrolladas con la cultura, tradiciones e identidad local se planteó a través de 3 indicadores (P9 a P11). Sus valores medios son estadísticamente superiores al punto medio de la escala empleada, situándose en torno al 7. Por tanto, parece que los residentes perciben un claro arraigo del turismo desarrollado, ya que las actividades turísticas reflejan la identidad local.

La endogeneidad del desarrollo turístico se establece a partir de 6 indicadores (P12 a P17). Todos los valores medios obtenidos son estadísticamente superiores al punto medio de la escala empleada, situándose por encima del 6, lo que pone de manifiesto que los residentes perciben la existencia de una estructura que permite retener los beneficios en la zona, utilizando y agregando valor a los recursos locales. El mayor valor medio obtenido (11, 27) se obtiene en "Las actividades turísticas se basan en el uso de los recursos naturales de la zona". (P15).

La complementariedad entre las actividades turísticas y las tradicionales de la zona así como la interacción entre turistas y residentes ha sido medida a través de 9 indicadores (P18 a P26).

Tabla 2. Descriptivos de las variables implicadas en el Turismo Rural Integrado.
Table 2. Descriptives of the variables involved in Integrated Rural Tourism.

Nº	Indicadores	Media	Moda	S.D
P1	En Internet hay suficiente información sobre las actividades de turismo rural que se desarrollan en la región	5,65	↑	5,00
P2	En las oficinas de turismo hay suficiente información sobre las actividades de turismo rural que se desarrollan en la región	6,40	↑	8,00
P3	Es fácil obtener información sobre las actividades de turismo rural de la región	5,99	↑	8,00
P4	Existe colaboración entre las empresas e instituciones de la zona para la organización de eventos, festivales, etc. (Fiesta de la Artesanía, de la Colonización, del Arroz, etc.)	6,60	↑	8,00
P5	En la región hay circuitos turísticos que unen las actividades de turismo rural de las distintas localidades	5,93	↑	8,00
P6	Las empresas e instituciones de la región promocionan conjuntamente las actividades de turismo rural	5,21	#	5,00
P7	Las empresas e instituciones de la región colaboran en el diseño de circuitos turísticos rurales	7,78	#	5,00
P8	El gobierno municipal, provincial y/o nacional colabora con las empresas e instituciones de la región para el desarrollo del turismo rural	5,86	↑	8,00
P9	Los festivales, eventos y/o fiestas de la región reflejan la cultura e identidad de la zona	7,73	↑	9,00
P10	Las actividades turísticas permiten al turista conocer la cultura y las tradiciones de la zona	7,19	↑	8,00
P11	Los nombres y/o imágenes locales se emplean en las marcas de los productos turísticos de la región	6,95	↑	8,00
P12	En general, las empresas turísticas son propiedad de personas nacidas en la región	6,09	↑	7,00
P13	En general, las empresas turísticas son gestionadas por personas nacidas en la región	6,36	↑	7,00
P14	Las actividades turísticas de la zona son financiadas por recursos económicos propios de la región	6,45	↑	8,00
P15	Las actividades turísticas se basan en el uso de los recursos naturales de la zona	8,24	↑	10
P16	Las actividades turísticas se basan en el uso de los recursos históricos de la zona	6,53	↑	8
P17	Las actividades turísticas se basan en el uso de los recursos culturales de la zona	5,97	↑	5
P18	Los servicios turísticos básico (alojamiento, comida, etc.) se complementan con actividades típicas de la zona (actividades de campo, visita a productores de productos regionales, etc.)	5,90	↑	8,00
P19	Los turistas pueden participar en actividades tradicionales o típicas de la zona	6,06	↑	7,00
P20	Las actividades turísticas se basan en las actividades típicas de la zona	5,93	↑	7,00
P21	Los servicios ofrecidos por las empresas turísticas incluyen alojamiento, comida y actividades típicas	5,61	↑	8,00
P22	Las empresas tradicionales productivas de la zona ofrecen servicios turísticos	5,01	#	5,00

↓ Estadísticamente por debajo del punto medio de la escala (5) (Con una confianza del 95%); ↑ Estadísticamente por encima del punto medio de la escala (5) (Con una confianza del 95%); # Estadísticamente igual al punto medio de la escala (5) (Con una confianza del 95%).

Fuente: Elaboración propia.

↓ Statistically below the mid point of the scale (5) (with a 95% confidence interval) ↑ Statistically above the mid point of the scale (5) (with a 95% confidence interval); # Statistically equal to the mid point of the scale (5) (with a 95% confidence interval).

Source: own elaboration.

Tabla 2 (cont.). Descriptivos de las variables implicadas en el Turismo Rural Integrado.
Table 2 (cont.). Descriptives of the variables involved in Integrated Rural Tourism.

Nº	Indicadores	Media	Moda	SD
P23	Los turistas comparten con los residentes el uso de lugares físicos de esparcimiento (plazas, playas, parques, etc.)	8,77	↑ 10,00	1,66
P24	Los turistas comparten con los residentes los mismos servicios públicos (sanidad, seguridad, etc.)	8,63	↑ 10,00	1,72
P25	Los turistas comparten con los residentes los mismos servicios de ocio (bares, restaurantes, etc.)	8,80	↑ 10,00	1,71
P26	Los residentes de la zona participan de las actividades, eventos y festivales turísticos	8,32	↑ 10,00	1,88
P27	El desarrollo turístico de la zona tiene en cuenta los intereses de sus residentes	5,87	↑ 5,00	2,67
P28	Las instituciones y/o empresas turísticas tienen en cuenta los intereses de los residentes al ofertar actividades turísticas	5,59	↑ 5,00	2,52
P29	Mis intereses influyen en las actividades turísticas que se ofrecen en la zona	5,09	# 5,00	3,12
P30	Existen los espacios adecuados para que los residentes manifesten sus opiniones con respecto a las decisiones turísticas	4,42	↑ 5,00	2,89
P31	...generan oportunidades de empleo para la comunidad	7,09	↑ 10,00	2,71
P32	...proporcionan beneficios a las empresas involucradas	8,01	↑ 8,00	1,90
P33	...generan beneficios a toda la comunidad	6,94	↑ 8,00	2,56
P34	...permiten mejorar las infraestructuras existentes	6,99	↑ 7,00	2,46
P35	...no incrementan el costo de la vida	7,12	↑ 10,00	2,82
P36	...ayudan a conservar la cultura e identidad de la región	7,53	↑ 8,00	2,13
P37	...mantienen la cultura tradicional	7,30	↑ 8,00	2,18
P38	...mejoran la apariencia de las ciudades y pueblos	7,54	↑ 8,00	2,00
P39	...aumentan las oportunidades de ocio	6,76	↑ 8,00	2,44
P40	...favorecen la conservación de los recursos naturales	6,77	↑ 8,00	2,32
P41	...incrementan la conciencia ecológica de la sociedad	6,49	↑ 7,00	2,42
P42	...favorecen el desarrollo de acciones dirigidas al mantenimiento del medio ambiente	6,45	↑ 8,00	2,54
P43	El turismo rural desarrollado en la región integra y beneficia a los actores involucrados al favorecer el desarrollo económico, social y la protección del medio ambiente	6,39	↑ 8,00	2,36

↓ Estadísticamente por debajo del punto medio de la escala (5) (Con una confianza del 95%); ↑ Estadísticamente por encima del punto medio de la escala (5) (Con una confianza del 95%); # Estadísticamente igual al punto medio de la escala (5) (Con una confianza del 95%).

Fuente: Elaboración propia.

↓ Statistically below the mid point of the scale (5) (with a 95% confidence interval) ↑ Statistically above the mid point of the scale (5) (with a 95% confidence interval); # Statistically equal to the mid point of the scale (5) (with a 95% confidence interval).

Source: own elaboration.

Los resultados muestran que, excepto uno, todos los valores medios obtenidos son estadísticamente superiores al punto medio de la escala empleada.

Se observa que existe una mayor valoración media para los ítems que indican el uso compartido de recursos por parte de residentes y turistas, siendo la variabilidad en las respuestas menor que en el resto de indicadores.

Por otro lado, los indicadores del diseño de productos turísticos complementarios a las actividades tradicionales muestran unas valoraciones medias en torno al 6, con la excepción del ítem P22 "Las empresas tradicionales productivas de la zona ofrecen servicios turísticos" que se sitúa en el punto medio de la escala. Por tanto, parece intuirse la existencia de dos vertientes claramente diferenciadas de la variable complementariedad en las percepciones de los residentes.

El empoderamiento se midió a través de 4 indicadores (P27 a P30).

Los resultados parecen indicar que no existe una alta percepción de la existencia de esta variable, ya que las valoraciones medias se sitúan en torno al punto medio de la escala empleada.

La mayor valoración media (5,87) se obtiene en P27 "El desarrollo turístico de la zona tiene en cuenta los intereses de sus residentes". Además, los residentes perciben una falta de medios a través de los cuales pueden manifestar sus opiniones con respecto al desarrollo turístico (P30, valor medio 4,42).

La sostenibilidad del turismo desarrollado en la zona fue medida a través de 12 indicadores: 5 de sostenibilidad económica (P31 a P35), 4 de sostenibilidad sociocultural (P36 a P39) y 3 de sostenibilidad medioambiental (P40 a P42).

En la tabla 2 (pág. 262-263), se muestra que todos los valores medios obtenidos se sitúan por encima del punto medio de la escala empleada. Por tanto, se observa una alta percepción de sostenibilidad económica, basada fundamentalmente en las percepciones correspondientes a la obtención de beneficios por parte de las empresas involucradas en el turismo (8,01), no incrementos del costo de la vida (7,12) y en la generación de oportunidades de empleo (7,09).

La existencia de sostenibilidad socio-cultural parece percibirse en menor medida. Así, los mayores valores medios reflejan la creencia de que el desarrollo turístico mejora la apariencia de los pueblos y ciudades (7,54) y ayuda a conservar la cultura e identidad local (7,53). Se percibe la existencia de sostenibilidad medioambiental, ya que sus indicadores muestran unos valores medios próximos al 6,5.

Finalmente, destacar que los residentes perciben un adecuado desarrollo del TRI en la zona (6,39).

Para operacionalizar las variables no observables, se procede a determinar sus dimensiones subyacentes. Para ello, se realizaron los correspondientes análisis de componentes principales con rotación *varimax*, y se analizó la fiabilidad de las escalas subyacentes detectadas a través de los correspondientes Alpha de Cronbach.

De los 42 indicadores inicialmente empleados para medir las variables no observables (A1 a A42), el análisis de fiabilidad a través de las cargas de cada indicador (relación de cada ítem con la variable no observable), lleva a la eliminación de 2 indicadores (P11 y P26).

En la tabla 3 (pág. 266-267), se muestran los resultados finalmente aceptados de los correspondientes análisis de componentes principales llevados a cabo, así como los análisis de fiabilidad de las escalas propuestas.

En la tabla 3 (pág. 266-267), se observa cómo la variable *Redes* está integrada por dos componentes que explican el 68,29% de la varianza (el 36,68% y el 31,61% respectivamente). El primer componente se denomina Redes Colaborativas (F1), ya que agrupa los ítems referidos a la existencia de colaboración, formal o informal, entre los agentes implicados, especialmente empresas e instituciones, en el desarrollo del producto turístico. El segundo componente se denomina Redes Informativas (F2), ya que refleja la existencia de distintas fuentes de información sobre el turismo de la zona así como la facilidad de acceso a la misma.

La fiabilidad de las escalas propuestas puede ser aceptada dado que los dos coeficientes Alpha toman valores superiores a 0,6 (56).

Los resultados correspondientes a la variable *Arraigo* (F3) verifican la estructura unidimensional de dicha variable, cuya componente explica el 82,86% de la varianza. En este caso, la fiabilidad de la escala se mide a través de la correlación entre los dos indicadores que la configuran.

Los resultados correspondientes a la *Endogeneidad* del desarrollo turístico, muestran la existencia de tres componentes que explican el 84,85% de la varianza. El primer componente (F4) (37,70% varianza) recoge los indicadores relativos a la propiedad, gestión y financiación de las actividades turísticas por parte de las personas de la región.

El segundo componente (F5) (30,18% varianza) recoge las actividades turísticas

basadas en los recursos históricos y culturales de la zona.

Finalmente, el tercer componente (F6) (16,97% varianza), integrado por un único indicador, recoge las actividades turísticas basadas en los recursos naturales de la zona.

Los análisis realizados verifican la fiabilidad de las escalas propuestas en las dos primeras componentes.

La variable *Complementariedad* está integrada por dos componentes que explican el 68,68% de la varianza. El primer componente (F7) (38,75% varianza) refleja la complementariedad entre las actividades tradicionales y las turísticas, mientras que el segundo componente (F8) (29,93% varianza) refleja el uso compartido de los recursos por turistas y residentes.

Los valores alpha obtenidos permiten aceptar la fiabilidad de las escalas.

La existencia de la variable *Empoderamiento* (F9) queda verificada.

Los resultados de la tabla 3 (pág. 266-267), muestran la existencia de una única dimensión que explica el 62,46% de la varianza. Esta variable recoge la influencia de los residentes en el desarrollo turístico. La fiabilidad de la escala y de los indicadores que la integran es aceptable.

Finalmente, se comprueba la dimensionalidad del concepto de sostenibilidad.

El análisis de componentes principales realizado muestra la existencia de tres componentes que explican el 71,25% de la varianza.

La primera componente refleja la Sostenibilidad Económica (F10) (26,14% varianza), el segundo la Sostenibilidad Sociocultural (F11) (22,97% varianza), y el tercero la Sostenibilidad Medioambiental (F12) (22,17% varianza).

La fiabilidad de las tres escalas de medida propuestas es aceptable.

Tabla 3. Resultados Finales Análisis Componentes Principales.
Table 3. Final Results of the Principal Components Analysis.

Indicadores	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
P1 En Internet hay suficiente información sobre las actividades de turismo rural que se desarrollan en la región		0,79										
P2 En las oficinas de turismo hay suficiente información sobre las actividades de turismo rural que se desarrollan en la región		0,88										
P3 Es fácil obtener información sobre las actividades de turismo rural de la región		0,87										
P4 Existe colaboración entre las empresas e instituciones de la zona para la organización de eventos, festivales, etc. (Fiesta de la Artesanía, de la Colonización, del Arroz, etc.)	0,67											
P5 En la región hay circuitos turísticos que unen las actividades de turismo rural de las distintas localidades	0,65											
P6 Las empresas e instituciones de la región promocionan conjuntamente las actividades de turismo rural	0,81											
P7 Las empresas e instituciones de la región colaboran en el diseño de circuitos turísticos rurales	0,80											
P8 El gobierno municipal, provincial y/o nacional colabora con las empresas e instituciones de la región para el desarrollo del turismo rural	0,71											
P9 Los festivales, eventos y/o fiestas de la región reflejan la cultura e identidad de la zona			0,91									
P10 Las actividades turísticas permiten al turista conocer la cultura y las tradiciones de la zona			0,91									
P12 En general, las empresas turísticas son propiedad de personas nacidas en la región				0,89								
P13 En general, las empresas turísticas son gestionadas por personas nacidas en la región				0,91								
P14 Las actividades turísticas de la zona son financiadas por recursos económicos propios de la región				0,76								
P15 Las actividades turísticas se basan en el uso de los recursos naturales de la zona						0,98						
P16 Las actividades turísticas se basan en el uso de los recursos históricos de la zona					0,94							
P17 Las actividades turísticas se basan en el uso de los recursos culturales de la zona					0,93							
P18 Los servicios turísticos básicos (alojamiento, comida, etc.) se complementan con actividades típicas de la zona (actividades de campo, visita a productores de productos regionales, etc.)							0,80					
P19 Los turistas pueden participar en actividades tradicionales o típicas de la zona							0,84					
P20 Las actividades turísticas se basan en las actividades típicas de la zona							0,80					
P21 Los servicios ofrecidos por las empresas turísticas incluyen alojamiento, comida y actividades típicas							0,73					

*Correlación / *Correlation

Fuente: Elaboración propia / Source: Own elaboration

Tabla 3 (cont.). Resultados Finales Análisis Componentes Principales.
Table 3 (cont.). Final Results of the Principal Components Analysis.

Indicadores	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
P22 Las empresas tradicionales productivas de la zona ofrecen servicios turísticos							0,76					
P23 Los turistas comparten con los residentes el uso de lugares físicos de esparcimiento (plazas, playas, parques, etc.)								0,87				
P24 Los turistas comparten con los residentes los mismos servicios públicos (sanidad, seguridad, etc.)								0,90				
P25 Los turistas comparten con los residentes los mismos servicios de ocio (bares, restaurantes, etc.)								0,88				
P27 El desarrollo turístico de la zona tiene en cuenta los intereses de sus residentes									0,86			
P28 Las instituciones y/o empresas turísticas tienen en cuenta los intereses de los residentes al ofertar actividades turísticas									0,88			
P29 Mis intereses influyen en las actividades turísticas que se ofrecen en la zona									0,65			
P30 Existen los espacios adecuados para que los residentes manifiesten sus opiniones con respecto a las decisiones turísticas									0,75			
P31 ...generan oportunidades de empleo para la comunidad										0,83		
P32 ...proporcionan beneficios a las empresas involucradas										0,79		
P33 ...generan beneficios a toda la comunidad										0,77		
P34 ...permiten mejorar las infraestructuras existentes										0,68		
P35 ...no incrementan el costo de vida										0,75		
P36 ...ayudan a conservar la cultura e identidad de la región											0,89	
P37 ...mantienen la cultura tradicional											0,84	
P38 ...mejoran la apariencia de las ciudades y pueblos											0,67	
P39 ...aumentan las oportunidades de ocio											0,43	
P40 ...favorecen la conservación de los recursos naturales												0,83
P41 ...incrementan la conciencia ecológica de la sociedad												0,92
P42 ...favorecen el desarrollo de acciones dirigidas al mantenimiento del medio ambiente												0,88
% de varianza	36,68	31,61	82,86	37,7	30,18	16,97	38,75	29,93	62,46	26,14	22,97	22,14
% de varianza acumulado	36,68	68,29	82,86	37,7	67,88	84,85	38,75	68,68	62,46	26,14	49,11	71,25
Alpha Cronbach	0,87	0,84	0,66*	0,83	0,80*		0,85	0,87	0,78		0,795	0,91

Correlación / *Correlation

Fuente: Elaboración propia / Source: Own elaboration

Una vez comprobada la existencia de las variables bajo estudio y sus respectivas dimensiones, se procede a determinar sus efectos sobre el desarrollo del TRI en la zona. Inicialmente, la hipótesis de partida es que las variables Redes (Colaborativas e Informativas), Arraigo, Endogeneidad (de propiedad de recursos, basada en recursos históricos-culturales, y basada en recursos naturales), Complementariedad (de actividades turísticas y tradiciones, y uso compartido de recursos por turistas y residentes), y Empoderamiento afectan positivamente al TRI. Para comprobar esta relación, se realiza un análisis de regresión a través de la aplicación del método *Enter*, siendo la variable dependiente "El turismo rural desarrollado en la región integra y beneficia a los actores involucrados al favorecer el desarrollo económico, social y la protección del medioambiente" (P43). Se recuerda que el análisis descriptivo inicialmente realizado mostraba una percepción positiva con respecto a esta variable por parte de los residentes (tabla 2, pág. 262-263).

Los resultados obtenidos correspondientes a la regresión realizada, mostrados en la tabla 4, reflejan que la capacidad explicativa de las

variables analizadas se sitúa en el 29,1%. Todas las variables analizadas, excepto las relacionadas con la Endogeneidad en cualquiera de sus componentes, el Arraigo y las Redes Colaborativas, ejercen un efecto positivo sobre la percepción del TRI.

Así, cuanto mayor sea el Empoderamiento de los residentes, la Complementariedad existente entre las actividades turísticas llevadas a cabo y las tradicionales de la zona, la existencia de Redes que informen sobre el turismo desarrollado en la zona y la necesidad de compartir recursos por residentes y turistas (lo cual repercute en la mejora de los mismos), mejor es la percepción que se posee sobre el desarrollo del TRI en la zona.

Dado que el desarrollo de un turismo sostenible constituye uno de los objetivos clave en el TRI, se procede a analizar la relación entre estas variables. De esta manera, se establece la existencia de una relación positiva entre las sostenibilidades económica, sociocultural y medioambiental con el desarrollo exitoso del TRI. Para testarla, al igual que en el caso anterior, se realiza un análisis de regresión a través de la aplicación del método *Enter*.

Tabla 4. Resultados Análisis de Regresión 1.

Table 4. Results of Regression analysis 1.

Variabes	Coefficiente Beta Estandarizado	t value	Significatividad
Constante		52,359	0,000
F1: Redes Colaborativas	-0,042	-0,633	0,527
F2: Redes Informativas	0,123	2,097	0,037
F3: Arraigo	-0,123	-1,742	0,083
F4: Endogeneidad Propiedad	0,010	0,154	0,878
F5: Endogeneidad Recursos Históricas y Culturales	0,060	0,911	0,363
F6: Endogeneidad Recursos Naturales	0,030	0,497	0,619
F7: Complementariedad Actividades Tradicionales y Turísticas	0,205	2,558	0,011
F8: Complementariedad Uso Compartido de Recursos	0,122	2,020	0,045
F9: Empoderamiento	0,403	6,155	0,000
R ² =0,316; R ² ajustado = 0,291			

Los resultados obtenidos, mostrados en la tabla 5, ponen de manifiesto el efecto positivo y significativo de las sostenibilidades en el TRI.

La capacidad explicativa de estas tres variables se sitúa en el 49,8%. El mayor peso lo ejerce la sostenibilidad medioambiental, seguido por la sociocultural y la económica.

Finalmente, dado que no se han establecido relaciones indirectas entre las variables, se procede a analizar los efectos de todas las variables analizadas sobre el TRI. Esto es, las variables Redes (Colaborativas

e Informativas), Arraigo, Endogeneidad (de propiedad de recursos, basada en recursos históricos-culturales, y basados en recursos naturales), Complementariedad (de actividades turísticas y tradiciones, y de uso compartido de recursos por turistas y residentes), Empoderamiento, Sostenibilidad Económica, Sostenibilidad Sociocultural y Sostenibilidad Medioambiental afectan positivamente al TRI.

Los resultados obtenidos, reflejados en la tabla 6, muestran que estas variables son capaces de explicar el 60,9% del TRI.

Tabla 5. Resultados Análisis Regresión 2.

Table 5. Results of Regression analysis 2.

Variables	Coefficiente Beta Estandarizado	t value	Significatividad
Constante		66,375	0,000
Sostenibilidad Económica	0,337	8,240	0,000
Sostenibilidad Sociocultural	0,362	8,865	0,000
Sostenibilidad Medioambiental	0,507	12,415	0,000
R ² =0,503; R ² ajustado = 0,498			

Tabla 6. Resultados de la Regresión 3.

Table 6. Results of Regression analysis 3.

Variables	Coefficiente Beta Estandarizado	t value	Significatividad
Constante		65,960	0,000
F1: Redes Colaborativas	-0,028	-0,525	0,600
F2: Redes Informativas	0,106	2,266	0,024
F3: Arraigo	-0,081	-1,396	0,164
F4: Endogeneidad Recursos Históricos y Culturales	-0,036	-0,717	0,474
F5: Endogeneidad Recursos Históricos y Culturales	0,071	1,327	0,186
F6: Endogeneidad Recursos Naturales	-0,025	-0,525	0,600
F7: Complementariedad Actividades Tradicionales y Turísticas	-0,032	-0,468	0,641
F8: Uso Compartido de Recursos	0,022	0,451	0,652
F9: Empoderamiento	0,213	3,892	0,000
Sostenibilidad Económica	0,315	6,972	0,000
Sostenibilidad Sociocultural	0,316	6,509	0,000
Sostenibilidad Medioambiental	0,483	10,401	0,000
R ² =0,63; R ² ajustado = 0,609			

Concretamente, las que ejercen un efecto positivo y significativo sobre el TRI, ordenadas por el peso de sus coeficientes beta estandarizados, son la Sostenibilidad Medioambiental, el Empoderamiento, Sostenibilidad Sociocultural, Sostenibilidad Económica y Redes Informativas.

Por tanto, se observa que la capacidad explicativa mostrada por las sostenibilidades diluye los efectos significativos de algunas de las variables que habían emergido cuando estas no habían sido incluidas. Los efectos mostrados por la variable Complementariedad, tanto entre las actividades turísticas y las tradicionales como por el uso compartido de los recursos, desaparecen (tabla 4, pág. 268). Este resultado pone de manifiesto la necesidad de analizar más detenidamente las relaciones que pueden existir previamente entre las variables analizadas las cuales afectarán al TRI. Esto es, se debe proponer un modelo que plasme las relaciones existentes entre las distintas variables analizadas, determinando así las interrelaciones existentes entre las mismas. Dicho modelo reflejaría el proceso a seguir para conseguir el adecuado desarrollo del TRI.

CONCLUSIONES

El turismo constituye una actividad económica que debería ayudar al desarrollo de determinadas zonas rurales. Dichas zonas deben poseer algún tipo de recurso sobre el cual planificar estratégicamente el desarrollo turístico perseguido. Estos recursos turísticos pueden ser cualquier elemento natural, objeto cultural o hecho social que pueda ser utilizado como causa suficiente para motivar un desplazamiento turístico. Por tanto, constituyen el principal compo-

nente del producto turístico, al determinar la selección del destino del viaje.

En el caso analizado, Tierra de Palmares, se cuenta con los recursos naturales, históricos y culturales necesarios para planificar una actividad turística que ayude al desarrollo de la zona. Pero, se debe tener en cuenta que, en líneas generales, el desarrollo turístico en un espacio rural debe tener un carácter extensivo que permita conservar la propia esencia del territorio. Es decir, se debe conseguir un desarrollo sostenible que permita la conservación de los recursos en los que se basa y la mejora de la calidad de vida de los residentes. Para ello, la integración de las distintas actividades resulta vital. Así, la cooperación entre los agentes implicados, la endogeneidad de los recursos empleados, el arraigo de las actividades así como su complementariedad con las actividades tradicionales de la zona deben ser los elementos clave a la hora de realizar la planificación estratégica de un proyecto turístico.

Esta planificación no puede ser llevada a cabo con éxito si no se tiene en cuenta la opinión de los residentes en la zona.

Comprender su perspectiva facilita la implantación de medidas que minimicen los efectos negativos del turismo y que maximicen sus beneficios (64), consiguiendo así un desarrollo turístico rural integrado.

En esta investigación se analizan las percepciones de la comunidad receptora, residentes en los departamentos Colón y San Salvador de Entre Ríos (Argentina), sobre las variables implicadas en el desarrollo del TRI.

Los departamentos de Colón y San Salvador, pertenecientes a la provincia de Entre Ríos, están ubicados en la región del Litoral de Argentina. La zona ofrece una experiencia turística diversa que

comprende varias tipologías de turismo entre las que destaca el natural y el rural, así como el cultural, gastronómico y el de salud y bienestar físico.

Las principales atracciones turísticas de la zona son las playas del río Uruguay, los balnearios de aguas termales, pequeños poblados rurales y el Parque Nacional El Palmar, una de las reservas naturales más importantes del país. Este turismo se centra en los recursos naturales y culturales locales, donde en un radio de 60 km se encuentran numerosos establecimientos de turismo rural y de naturaleza tales como granjas, bodegas, posadas de campo, almacenes rurales, centros de producción artesanal de quesos, licores, chacinados y otros productos locales. Estancias de producción de nueces, refugios naturales donde pueden realizarse avistaje de aves y animales autóctonos, pesca deportiva y cabalgatas. También son muy importantes los festivales y las tradiciones populares, tales como la Fiesta Nacional de la Artesanía, la Fiesta de la Colonización, la Fiesta Nacional del Arroz, el Festival Provincial de Danzas, entre otras.

Las investigaciones realizadas parecen indicar que las actitudes de los residentes hacia el turismo son más favorables en las primeras fases de desarrollo de un destino (81). Esta parece ser la situación en el caso analizado, ya que en el momento de realizar el estudio, existe una percepción positiva del turismo desarrollado hasta ese momento.

El análisis descriptivo realizado muestra que los residentes perciben una alta existencia de complementariedad y arraigo. La complementariedad se percibe desde dos vertientes: la existente entre las actividades turísticas y las tradicionales de la zona, y la referida al uso compartido de lugares y servicios por turistas y residentes. Los indicadores de uso

compartido de lugares físicos de esparcimiento, servicios públicos y de ocio por residentes y turistas son los que reciben mayor valoración. Por tanto, no se percibe una alta carga en dichas infraestructuras y servicios, lo cual puede ser debido a que la presencia de turistas representa un beneficio para la comunidad local, ya que los residentes pueden también disfrutar de los servicios turísticos ofertados a los turistas. Además, los ingresos obtenidos por las empresas en temporada alta les permite mantener los servicios durante todo el año.

Por otro lado, la complementariedad entre actividades turísticas y tradicionales es ligeramente superior a la media, existiendo establecimientos que combinan actividades tradicionales productivas con actividades turísticas (ej. estancia de producción de nueces, bodega, establecimientos productivos de quesos, chacinados, licores).

También queda patente el relativamente alto grado de arraigo percibido existente. Por las características propias del tipo de turismo desarrollado en la región, este se encuentra altamente ligado al entorno donde se lleva a cabo, a la cultura, tradiciones e identidad local. Este arraigo puede también ser explicado por la antigüedad de los residentes en la zona.

La gran mayoría han estado viviendo en la zona por más de 15 años y más de la mitad por más de 25 años, lo cual les confiere un mayor sentimiento de identidad sociocultural y de pertenencia a un grupo. Tienen conciencia del valor que poseen los activos intangibles de la región, favoreciendo la integración de la economía y de la sociedad. Asimismo, los turistas que visitan la zona tienden a ser aloécnicos, motivados por nuevas experiencias, destinos diferentes situados fuera de los circuitos convencionales.

Este tipo de turistas suele mantener un alto nivel de actividad y se arraiga en su destino (63).

Los indicadores de endogeneidad reciben una puntuación levemente superior a la media. En la última década se está produciendo una llegada de personas de otras ciudades, en especial de la capital del país, con el objetivo de instalar empresas turísticas.

A pesar de este componente que puede ser percibido como no endógeno por muchos residentes por tratarse de personas no nacidas en la región, en su mayoría se trata de pequeños emprendimientos familiares y no de grandes cadenas que se llevan los ingresos generados fuera de la región. Por tanto, a pesar de esta moderada puntuación de endogeneidad, los residentes perciben que los beneficios generados se retienen en las localidades donde se produce.

Las variables cuyos indicadores parecen ser percibidos que existen en menor medida son Redes y Empoderamiento, por lo que parecen emerger como las principales restricciones a la formación de un TRI fuerte en la región. A pesar de los esfuerzos realizados en la micro región "Tierra de Palmares" las redes tanto informativas como colaborativas no están suficientemente desarrolladas, especialmente en el desarrollo de circuitos turísticos y en la promoción conjunta de actividades. Esta barrera a la conexión e integración del destino coincide con los resultados encontrados por Ilbery, Saxena y Kneafsey (2007) quienes explican que cuando la tradición del turismo es limitada es difícil desarrollar un sentimiento de bien común que trascienda las barreras de del individualismo y el interés propio.

Las empresas y organizaciones prefieren competir a cooperar ya que no son conscientes de los potenciales

beneficios de la colaboración. El sector público debe apoyar el desarrollo y expansión del turismo tanto a nivel macro como microeconómico. Pero, para que la planificación estratégica del gobierno nacional sea efectiva es necesaria la colaboración de los gobiernos provinciales y los municipios, ya que garantiza la implementación de las actividades planificadas y constituye una herramienta para que los habitantes puedan controlar las iniciativas, las decisiones y los recursos (7).

Por último, se percibe un escaso nivel de Empoderamiento en la región, ya que los residentes no perciben que sus intereses influyan en el desarrollo turístico y no encuentran que existan los espacios para manifestar sus opiniones respecto a este tema. Este es otro importante límite al desarrollo de un turismo integrado.

Sin embargo, se percibe positivamente la existencia de un turismo sostenible.

La sostenibilidad económica es la que se percibe en mayor medida, seguida por la sociocultural y la medioambiental. Así, se perciben positivamente los beneficios obtenidos por las empresas turísticas, y en menor medida la mejora de los servicios e infraestructuras públicas debido al desarrollo turístico. Esto último puede deberse a que algunas infraestructuras, por ejemplo los caminos, no han experimentado una mejora concordante con el desarrollo turístico de los últimos años.

Estas percepciones quedan corroboradas al comprobar la existencia de las variables bajo estudio, las cuales deben ser adecuadamente planificadas y ejecutadas para alcanzar un TRI. Así, queda claro cómo el turismo desarrollado debe ser percibido como sostenible, en sus dimensiones económica, social y medioambiental, para poder alcanzar el TRI. La conservación y el mantenimiento de los recursos naturales y socioculturales a partir de los cuales se

desarrolla la actividad turística resulta clave para alcanzar el objetivo de sostenibilidad medioambiental y sociocultural. Para ello, se debe incrementar la conciencia ecológica de la sociedad y mejorar la apariencia de las ciudades y pueblos. Estos objetivos de sostenibilidad no deben ser incompatibles con la mejora de la situación económica de la región. Por el contrario, deben ayudar a generar oportunidades de empleo y beneficios empresariales que redunden en toda la comunidad.

Pero este desarrollo sostenible no es posible si no se consigue la integración de todas las actividades y actores implicados. Esta integración es percibida a través de la existencia de redes, especialmente informativas, de la complementariedad entre las actividades tradicionales de la región y las turísticas, del uso de recursos compartidos por parte de residentes y turistas, y especialmente, por proporcionar a los residentes los medios a través de los cuales se sientan parte integrante del proceso de decisión. El empoderamiento de los residentes resulta clave para alcanzar los objetivos del TRI.

En conclusión, en términos de recursos naturales, socioculturales y servicios ofrecidos, la región tiene un gran potencial de desarrollo en términos de TRI, pero los residentes perciben que no se está alcanzando todo su potencial debido principalmente a la falta de cohesión y de colaboración entre empresas e instituciones públicas y privadas y a la escasa participación de los residentes en la toma de decisiones turísticas. Esto es especialmente importante cuando se analizan empíricamente los efectos de las percepciones de las variables analizadas en el desarrollo del TRI en la región. El análisis de regresión realizado muestra el efecto positivo que ejercen las percepciones de sostenibilidad en sus tres dimensiones (económica, sociocultural

y medioambiental), de empoderamiento y de redes informativas en la percepción positiva del desarrollo turístico alcanzado. Por tanto, si se desea la participación de los residentes en el desarrollo exitoso del turismo, es necesario gestionar adecuadamente estas variables.

Las redes son vitales para el desarrollo y consolidación de un destino turístico rural y son el punto de partida del proceso que lleva al desarrollo de un Turismo Rural Integrado. La administración de la micro región turística "Tierra de Palmares" debe concentrar sus esfuerzos en desarrollar las conexiones entre empresas e instituciones.

Los municipios son los responsables de la gestión y de la integración, ya que deben llevar a cabo la conservación, puesta en valor turístico y promoción de los activos turísticos tanto para la obtención de inversiones como para la conquista del mercado (47). Para esto, la administración pública debe, en primer lugar, aumentar la cantidad de organizaciones que se unen a las redes debido a los beneficios que estas pueden conseguir y, en segundo lugar, impulsar la formación de redes formales a través de la evolución gradual de agrupaciones informales en estructuras más concretas (33). Para lo primero, se debe comunicar con claridad los beneficios concretos que la integración traerá para cada una de las organizaciones miembros de la red a fin de incentivar su inclusión. Para lo segundo, promover la formación de redes formales entre empresas e instituciones públicas con fines específicos, por ejemplo comisiones para la organización de festivales, circuitos turísticos que unan puntos de distintas localidades, campañas de promoción conjunta, entre otras.

Simultáneamente, la administración pública debe proporcionar información colectiva de la región. La percepción que tengan los residentes sobre la información

que se está llevando a cabo mejora su percepción sobre el desarrollo turístico realizado y, por lo tanto, su disposición a actuar de manera favorable.

Actualmente "Tierra de Palmares" cuenta con un sitio web con información de servicios y actividades de cada una de las localidades que conforman la región. Pero es difícil encontrar físicamente esta información colectiva, ya que por lo general las oficinas turísticas ofrecen folletos e información solo de su localidad. Esta mejora traería importantes beneficios ya que favorecería el intercambio de turistas entre las ciudades y pueblos. Asimismo, favorecería el desarrollo de una experiencia turística completa, innovadora y coordinada, incrementando la satisfacción de los turistas.

Es necesario el apoyo del Estado para desarrollar la línea estratégica establecida en el PEDTS relacionada con el marketing. Así se debe ayudar a posicionar a la micro región como un destino turístico con diversidad de productos, calidad en la prestación de servicios y satisfacción de las necesidades de los turistas (7).

Junto a lo anterior, se deben favorecer los procesos de participación ciudadana en el proceso de toma de decisiones del desarrollo turístico. En este aspecto, destacar que el departamento de Colón

posee un carácter pionero tal y como refleja Medina (2008).

La investigación desarrollada pone de manifiesto una importante línea de investigación futura centrada en el análisis detallado de las relaciones existentes entre las variables bajo estudio. Este conocimiento permitiría a los gestores turísticos ser conscientes de la complejidad de las relaciones, de la diversidad de agentes y recursos que intervienen en el desarrollo del turismo rural y de cuáles son los elementos esenciales que favorecen el desarrollo del turismo en forma sostenible, a través de un marco de análisis y gestión integral. En definitiva, generar un destino turístico que pueda ser disfrutado por residentes y visitantes.

Un turismo en el que se refuercen las conexiones entre empresas, instituciones e individuos, que mantenga las tradiciones locales, que se ha adecuado a las estructuras existentes, que acompañe a las actividades productivas tradicionales, que se lleve a cabo con sus propios recursos reteniendo los beneficios en la región, que proteja los recursos naturales, que se lleve a cabo con la participación y compromiso de la comunidad local, atendiendo a sus demandas e intereses, y por tanto, que tienda a mejorar el sistema económico, sociocultural y ambiental de la comunidad como un todo.

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Measuring market integration and pricing efficiency along regional maize-tortilla chains of Mexico

Análisis de la transmisión de precios en las cadenas regionales de comercialización de tortillas de maíz en México

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ABSTRACT

When market performance is analyzed, a key issue to understand is the extent to which different agents in the supply chain respond to changes in price shocks. This paper explores the Mexican maize-tortilla chain through a price transmission analysis. It focus on a country-level study of the Mexican tortilla market by considering different regions and commercialization chains. Using a Vector Error Correction Model (VECM) the integration of the Mexican maize tortilla market between the inter-region *tortillería*, inter-region supermarket and intra-region *tortillería*-supermarket prices are investigated. The findings suggest that price transmission between *tortillerías* and supermarkets within a region seems to have triggered a shift in some consumers' place of purchase. Moreover, it demonstrates that cointegration level mainly depends of storage, logistics and transportation that could be the reason for the upsurge in variability of tortilla prices between states. These results appear to validate that radial transportation system in Mexico has benefited the central regions at the expense of the periphery regions, thus enabling *tortillerías* in Central regions to remain competitive with supermarkets and retain their market share.

Keywords

maize tortilla industry • Mexico • price transmission • supply chain

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RESUMEN

Cuando se analiza el comportamiento de los mercados, un elemento clave es entender hasta qué punto los diferentes agentes de la cadena de comercialización responden a los cambios en las perturbaciones de los precios. Este trabajo estudia las cadenas de comercialización de tortillas de maíz en México, a través, de un análisis de transmisión de precios. Este producto es una de las principales fuentes alimenticias en México. Este estudio analiza diferentes regiones y cadenas de comercialización utilizando un Modelo de Corrección de Errores Vectoriales (VECM) para investigar la integración del mercado mexicano de tortillas de maíz entre diferentes agentes comercializadores. Estos agentes son la tortillería interregional, el supermercado interregional y los precios intra-regionales de la relación tortillería-supermercado. Los resultados sugieren que la transmisión de precios entre las tortillerías y los supermercados dentro de una región parece haber provocado un cambio en el lugar de compra de algunos consumidores. Lo anterior, demuestra que el nivel de cointegración depende principalmente del almacenamiento, la logística y el transporte, lo que podría ser la razón del aumento en la variabilidad de los precios de la tortilla entre estados. Estos resultados parecen validar que el sistema de transporte radial en México ha beneficiado a las regiones centrales a expensas de las regiones periféricas, permitiendo así que las tortillerías de las regiones centrales sigan siendo tan competitivas como los supermercados, permitiendo la conservación de sus cuotas de mercado.

Palabras clave

tortillas de maíz • México • transmisión de precios • cadena de suministro

INTRODUCTION

Tortilla is the most important Mexican consumer good. It represents 38% of the protein, 45% of the calories and 49% of the calcium among the Mexican population's dietary intake (23). Maize is the traditional source of grain for this product and it is the most widely crop produced in Mexico.

Mexico produces 23 million t of maize and ranks fourth in producing countries in the world, after the United States, China and Brazil, with 224, 146 and 42 million t respectively (1). The maize tortilla industry plays a significant role in the Mexican economy, accounting for 1% of Mexico's GDP. The maize-tortilla industry directly employs about 225,000 workers in mills, flour factories, and *tortillerías* in the production of maize flour, maize dough (*nixtamal*), and maize tortillas (17). There are more than 78,000 *tortillerías* (tortilla bakeries) and mills distributed

throughout México, employing over 2 million agriculture workers in the maize productive sector (7, 34).

There are two main processes in the production of maize tortilla: maize flour and *nixtamal* (maize dough). The maize flour industry is highly concentrated, being their transportation and storage operations subsidized by the Mexican government in order to maintain tortilla prices stable (28). In contrast, the *nixtamal* industry is highly decentralized and usually not vertically integrated to the storage, logistics, and distribution of maize (31). In the case of mills and *tortillerías* they acquire maize from commercializing firms, who buy directly from producers, distribute and store maize in urban places, and sell to the *nixtamal* industry at higher prices, thus causing this type of tortillas to be costlier (35).

The maize tortilla value chain presents an important barrier to the entry for new competitors due to the high level of concentration in imports, storage and marketing of maize (34). Gasca (2014) suggested that government subsidies along the maize-tortilla value chain benefited large maize commercializing firms, which used their market power to finance speculative operations of storage, fleet, and export. Thereby exacerbating the effects of the rise in the international price of maize in the domestic market by causing price distortions along the maize-tortilla value chain. The tortilla demand is very inelastic to a change in price and it is considered to behave as a normal inferior good (21, 30). According to Wood *et al.* (2012), the income elasticity of demand for tortilla by non-poor and poor households is not significantly different from zero except for the urban non-poor who have a very small income elasticity, indicating that changes in income are unlikely to change tortilla demand. This is a reflection of the relevant status of tortilla as a staple that these households consume in the desired quantity. Therefore, low income household's standard of living has suffered the most as a consequence of price peaks events, such as, the tortilla crisis happened in 2007 (38).

From the supply side, *tortillerías* and supermarkets mainly distribute tortillas. The first have increased dramatically after the deregulation of the tortilla industry in 1998 (37). However, due to the highly inelastic demand for the consumption, its price has not decreased as would be expected in a competitive market but instead it has increased (23). Following Keleman *et al.* (2009), this situation could be exacerbated by the current public programs that incentivize the opening of new *tortillerías* and mills through subsidies.

The second player, supermarkets, accounted in 2014 for the distribution of 25% of the total maize tortillas commercialized in Mexico and they have emerged as a serious competitor of the traditional *tortillerías* (27). Supermarkets have a greater presence in northern and central parts of Mexico, having less presence in southern Mexico and rural areas (25). Throughout the last decade, regular food purchases at supermarkets of middle and high-income households have increased, this is reason to believe that at a medium-term supermarkets will consolidate their position as the primary competing channel of *tortillerías*, acquiring greater market share of tortilla sales at the expense of them (10).

With such a broad panorama, it should not be surprising that farmers and producers behave in different following the socioeconomic environment of their regions, therefore influencing the market performance along the supply chain of maize tortilla. This is due largely to asymmetries among segments of this chain, which conditioned the responses of industrial-scale corporations and small-scale family businesses.

When the performance of agricultural markets are analyzed, a key issue is the extent to which the different agents in the supply chain respond to changes in price shocks (29, 36). Therefore, the analysis of spatial price transmission is central in understanding the extent of the integration of economic agents into the market process (16). While studies of price transmission in a spatially separated contexts have received substantial attention in the literature [*e.g.* Ethiopia (12); Ghana (6) and Chile (35)], only a few studies have explicitly examined the impact of market chain structures on the transmission of price signals among regions.

The paper aims to fill this gap by exploring the performance of the Mexican maize-tortilla chain through a regional price transmission analysis. In particular, focus on a country-level study of the Mexican tortilla market by considering different regions and commercialization chains. Innovates by implement a Vector Error Correction Model (VECM) to determine the spatial price integration level of inter-region *tortillería* price, inter-region supermarket price and the intra-region *tortillería*-supermarket price. Since these goods should trade freely between the different regions in Mexico, it is hypothesized that tortilla price should be positively cointegrated with in their producing regions and between the two main selling points, *tortillerías* and supermarkets.

The article proceeds as follows: Sections 2 describes the data sources and methodology. Section 3 presents the results and discussion, respectively. Finally, Section 4 summarizes the main conclusions.

MATERIALS AND METHODS

Data description

A total of 362 weekly observations from the January 2007 to December 2013 were used to implement the VECMs (figure 1). Tortilla price series were acquired from the SNIIM (National Information System and Market Integration) (33) for each of the 50 urban centers spread throughout all of Mexico.

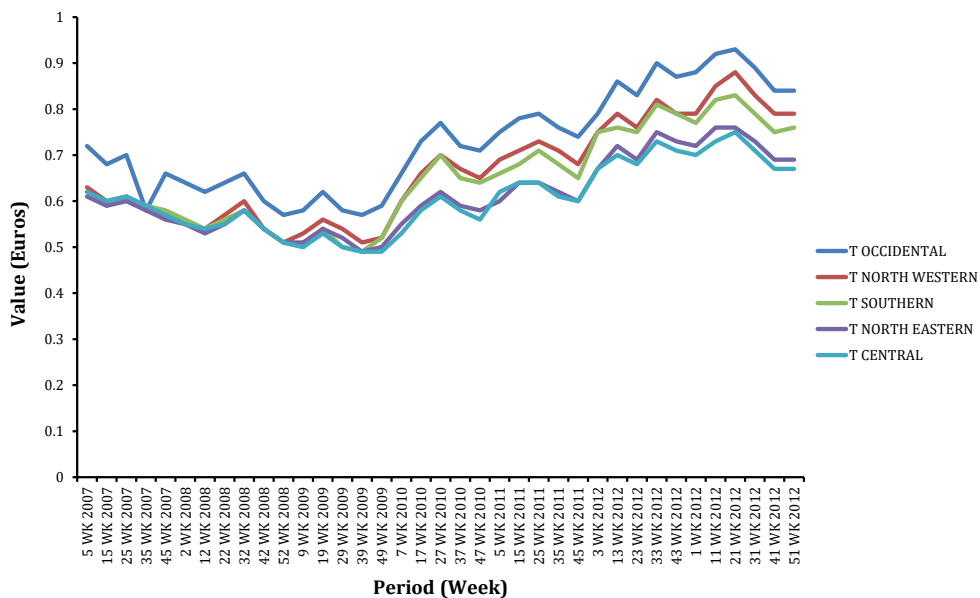


Figure 1. Average prices of tortilla for the 5 regions considered on this study (expressed in Euros).

Figura 1. Precios promedio de tortilla para las 5 regiones consideradas en este estudio (expresadas en Euros).

In order to classify each price pair according to their sales channels (supermarket and *tortillería*), the ENIGH 2006 and ENIGH (National Survey of Household Income and Expenditure) 2012 surveys were used to compare the percentage share of tortilla purchases consumers made at each channel.

Price data was transformed to the historic Euro value because price fluctuations distort the value of economic values measured in nominal values (32). In this sense, since there is no daily or weekly consumer price index measurement, transformation to Euros was decided over the dollar or Mexican Peso given to the lower inflation level in the European Union during the time being considered (13).

All price series were transformed to natural logarithms to enable the coefficient's interpretation as elasticity. The Mexican urban centers were identified following the classification of the Mexican Secretariat of Agriculture, Livestock, Fisheries, Rural Development, and Food (SAGARPA). These centers were sorted by regions according to their distance and population level (17).

The regions are: North West, North East, Occidental, Central and Southern Distinct variables for tortilla (from items bought) and for regions (from chain

variable containing state information). The sample was filtered for tortillas that were bought in urban centers of more than 100,000 habitants and in the respective region. This allowed a deeper analysis regarding the proportion of urban household tortilla purchased by supermarkets or *tortillerías* for the respective region and year (table 1).

Price transmission analysis

Well integrated markets play an essential role in reducing risks caused by supply and demand shocks by facilitating adjustments in trade flows across space, and in doing so reducing price variability faced by consumers and producers (4).

Market integration can be measured through short and long run price transmission elasticity, which measures the effect of a price change for a good in one market of the price in another market (39).

Most of price transmission research in Mexico has been centered on analyze the effect of international price shocks on Mexican maize markets and the dynamic of vertical price transmission along the tortilla industry. For example, Motamed *et al.* (2008) analyzed the price transmission between US yellow maize and Mexican white maize.

Table 1. Average distance and population for each region considered on this study.

Tabla 1. Distancia promedio y población para cada región considerada en este estudio.

Av. Distance between regions	North Western	North Eastern	Occidental	Central	Southern	Population
North Western	N/A	1091	1442	1776	2300	10,307,316
North Eastern	1091	N/A	736	1136	1623	17,200,470
Occidental	1442	736	N/A	448	935	23,437,097
Central	1776	1136	448	N/A	601	38,807,720
Southern	2300	1623	935	601	N/A	22,583,935

Source: INEGI (2014) / Fuente: INEGI (2014)

Their findings demonstrated a weak evidence of cointegration between these two prices, further suggesting that public subsidy programs could affect the convergence of price shocks between them. Araujo-Enciso (2011) studied the vertical price transmission for the Mexican Tortilla Industry by analyzing how social capital influenced price transmission between maize, maize flour, and maize tortilla. The work that most resembles this analysis was done by Appendini and Quijada (2010) who analyzed the production and consumption strategies of staple food (maize-tortilla) by rural households of Mexico.

This work explores spatial price transmission of maize tortilla for 5 regions distributed throughout Mexico by considering the most important urban centers and the supermarket channels. To estimate the speed of price transmission between regions, an approach based on Engle and Granger (1987) was used. Two price series P_t^A and P_t^B from regions A and B respectively are cointegrated if each is a non-stationary process (integrated of order 1 or I(1)) and if the residuals ε_t of the long-run relationship between these prices are stationary (integrated of order 0 or I(0)).

$$p_t^A = \beta_0 + \beta_1 p_t^B + \varepsilon_t \quad (1)$$

Before the price transmission model can be modeled, stationarity, cointegration level and long run cointegration need to be verified. The Leybourne (1995) and Otero and Smith (2012) critical values were applied to test the stationarity for each price series. When the existence of unit roots are confirmed, it is possible to

formulate a test for cointegration vectors, that is, the long run equilibrium relations. In general terms, in the presence of two variables y_t and x_t which are I (1) the residuals will be I (1).

However, if these variables are I(0), y_t and x_t are called cointegrated. The cointegration level between series was ranked according to the Johansen trace method (18) and the optimal lag was decided by the Akaike Criterion and Final Prediction Error (21). The test results are presented in table 2 (page 285). Assuming that P_t^A and P_t^B are indeed cointegrated, a vector error correction model (VECM) for these two prices are estimated. The VECM takes the following general form equation 2*.

The adjustment parameters α_p^{AB} and α_p^{BA} measure the speed with which price transmission takes place. Hence, they are the parameters of interest in the following stages of this analysis. Error correction, and thus cointegration between P_t^A and P_t^B , requires that $\alpha_p^{AB} < 0$ and $\alpha_p^{BA} > 0$. For example, if P_t^A is too high with respect to P_t^B then $\hat{\varepsilon}_t^*$ will be positive. In this case, and in equation (2) ensure that p^A falls and p^B increases in the next period, thus driving these prices closer to the long-run relationship. **Overall, the long-run price transmission elasticity (β_1) and short term error corrections terms (α_1 & α_2) for *tortillería* prices between regions (3 pairs), supermarket prices between regions (2 pairs) and finally, between supermarket and *tortillería* prices with in regions (2 pairs) were estimated. Therefore, 7 VECM's were implemented by using price pairs corresponding to each *tortillería*-supermarket and regional centers interactions (table 3, page 285).**

$$\begin{aligned} * \quad \Delta p_t^A &= \gamma_0 + \sum_{i=0}^2 \gamma_i^B \Delta p_{t-1}^B + \sum_{i=1}^2 \gamma_i^A \Delta p_{t-1}^A + \sum_{p=0}^3 \alpha_p^{AB} \hat{\varepsilon}_{t-1}^* + v_t^A \\ \Delta p_t^B &= \delta_0 + \sum_{i=0}^2 \delta_i^A \Delta p_{t-1}^A + \sum_{i=1}^2 \delta_i^B \Delta p_{t-1}^B + \sum_{p=0}^3 \alpha_p^{BA} \hat{\varepsilon}_{t-1}^* + v_t^B \end{aligned}$$

Table 2. Cointegration ranks according the Johansen Trace Method (18).

Tabla 2. Rankings de cointegración de acuerdo con el Método de Johansen (18).

Region	Rank	Eigen value	Trace Test	P-value
T. North Western - T. North Eastern	0	0.1318	57.741	0.0000
	1	0.0183	6.689	0.3886
T. North Western - T. Southern	0	0.1251	55.981	0.0000
	1	0.0211	7.738	0.2825
T. North Eastern - T. Southern	0	0.1387	60.342	0.0000
	1	0.0176	6.433	0.4179
S. Occidental - S. Southern	0	0.1055	45.259	0.0000
	1	0.0141	5.118	0.5871
S. Occidental - S. Central	0	0.1540	66.266	0.0000
	1	0.0161	5.8813	0.4858
T. Central - S. Central	0	0.0376	14.890	0.0172
	1	0.0028	1.0174	0.3647
T. Occidental - S. Occidental	0	0.0493	18.004	0.0189
	1	0.0008	0.2873	0.5919

Table 3. VECM Results for the Bivariate Cointegration.

Tabla 3. Resultados del Modelo Vectorial de Correccion de Errores.

Interactions	Variables	β_1	α_1	p-value	α_2	p-value	Durbin Watson Test	Dist. Reg. (Km)
Inter-regional Supermarket Cointegration	$\Delta \text{LogP}^{\text{Central}} / \Delta \text{logP}^{\text{Occidental}}$	-0.8626	-0.1258	0.0024 ***	0.1552	0.0002 ***	1.8698	448
	$\Delta \text{LogP}^{\text{Southern}} / \Delta \text{logP}^{\text{Occidental}}$	-0.8995	-0.0361	0.3982	0.1679	0.0001 ***	1.8213	935
Inter-regional Tortillería Cointegration	$\Delta \text{LogP}^{\text{Southern}} / \Delta \text{LogP}^{\text{NorthEastern}}$	-0.9178	-0.1956	0.0009***	-0.0776	0.2198	1.8932	1623
	$\Delta \text{LogP}^{\text{Southern}} / \Delta \text{LogP}^{\text{NorthWestern}}$	-0.9867	-0.2178	0.0001***	-0.0867	0.1756	1.8390	2300
	$\Delta \text{LogP}^{\text{NorthWestern}} / \Delta \text{logP}^{\text{NorthEastern}}$	-0.9156	-0.1810	0.0075***	0.0687	0.2287	1.8817	1091
Intra-regional Tortillería-Supermarket Cointegration	$\Delta \text{LogP}^{\text{T.Occidental}} / \Delta \text{logP}^{\text{S.Occidental}}$	-0.4976	-0.0886	0.0003***	-0.0390	0.2561	1.8516	N/A
	$\Delta \text{LogP}^{\text{T.Central}} / \Delta \text{LogP}^{\text{S.Central}}$	-0.5567	-0.1056	0.0002 ***	-0.0701	0.0886	1.9984	N/A

RESULTS

Observing table 4 (page 286) the average sale prices of tortillas at supermarkets are considerably lower than *tortillerías* in all regions. When both mean prices are compared, it was found that

supermarket prices between regions are smaller than *tortillería* prices, further suggesting higher volatility due to the atomization of these last sale points.

Table 4. Time series avg. price in Euros for period January 2007 - December 2013.**Tabla 4.** Precio promedio en Euros para el periodo enero 2007 - diciembre 2013.

Region & Point of Sale	Avg. Price in Euros	Standard Deviation
Tortillería_Occidental	0.615	0.077
Tortillería_North Western	0.733	0.105
Tortillería_Southern	0.664	0.105
Tortillería_North Eastern	0.652	0.098
Tortillería_Central	0.608	0.072
Supermarket_Occidental	0.438	0.101
Supermarket_North Western	0.456	0.100
Supermarket_Southern	0.435	0.099
Supermarket_North Eastern	0.459	0.112
Supermarket_Central	0.429	0.095

Source: SNIIM (2014) / Fuente: SNIIM (2014)

The Leybourne (1995) and Otero and Smith (2012) critical values confirmed that 12 of the 25 pairs and 15 of 25 pairs are cointegrated of order 1, respectively. For all price pairs, the Durbin Watson statistics are both close to 2, meaning that there is low auto-correlation of the residuals, all results are available upon request. These findings confirm a long-term bivariate cointegration relationship in the following pairs: *Tortillería* Central-SupermarketCentral, *Tortillería* Occidental-Supermarket Occidental, *Tortillería* North Eastern-*Tortillería* Southern, *Tortillería* North Western-*Tortillería* Southern, *Tortillería* North Western-*Tortillería* North Eastern, Supermarket Central-Supermarket Occidental, Supermarket Occidental-Supermarket Southern. The Johansen trace test method confirmed a bivariate cointegration rank 1 for 7 price pairs. These results are presented on table 2 (page 285).

With respect to the VECM estimation results, the cointegration parameters for each pair of regional interactions are presented in table 4.

DISCUSSION

Inter-Regional Supermarket

For the Occidental-Central price pair, the long-term elasticity (β_1) was 0.86, that is, a 1% increment in the price of Central region would generate an average increment of 0.86% in the prices of the Occidental region. Both short-term error correction terms indicate a proper (negative) and significant adjustment toward equilibrium represented by α_1 (-0.13) and α_2 (0.15). These results demonstrate that prices between Occidental and Central regions follow each other, being the Central region's α_1 the dominant price maker, since it corrects less than the Occidental region.

In contrast with β parameters, the average lower correction time toward equilibrium of Central-Occidental is consistent with the negative impact of distance.

Despite its shorter avg. distance (435 km), the Central-Occidental pair has the lower β_1 (0.86) with respect to the Occidental-Southern pair, which presents a β_1 of 0.90 and a distance between them of 935 km. This is not consistent with

the concept that distance should have a negative impact on price transmission. According to ASERCA (3), the way supermarkets determine their prices could be a factor in the asymmetric relation between price transmission and distance. For example, in most of Mexican supermarkets, the consumer price is determined by their regional headquarters, which define an average of 40% margin independent of the localization of each one (28). This could generate a relative market segmentation, which seem to have triggered a shift in the consumer preferred place of purchase and a lack of cointegration within the Southern, North Western and North Eastern regions.

Following Araujo-Enciso (2011), Mexican supermarkets have seen their market share increased, while corner stores and street vendors have seen their joint market share reduced. The factors underlying this situation are diverse. First, they show an increasing consumption of tortilla among urban areas. In these areas, the supermarkets concentrate the sales of tortilla, thus generating a trade-off effect with respect to the rest of sale's points (14). Second, regarding prices of tortillas, maize participation in the cost structure of the development of satellite tortilla mass dough is over 68% as opposed to maize meal pathway (5). As a result, the increase in the price of maize flour or dough is reflected directly in the price of tortillas sold in *tortillerías* and supermarkets. Due to the greater bargaining power of the supermarkets, they obtain more attractive customer prices in relation to corner stores and street vendors.

On the other hand, Keleman et al (2009) demonstrated that supermarkets would acquire greater market share as the overall *tortillería* prices remained high and supermarket prices remained low. Of the findings, it is expected that supermarket

increases their market power allowing a more intense participation of the overall maize-flour industry on these sale points. Although not all supermarket prices present evidence of cointegration between regions, the difference in regional prices is small when compared to the range of average *tortillería* prices. This can be attributed to the vertical integration of flour companies in buying grain directly from producers on contract, grain storage, and logistical distribution. They have many plants strategically distributed throughout Mexico and buy directly from producers, store and distribute grains to their own facilities and also sell maize at a premium to the nixtamal industry (27). In addition, supermarkets have the financial resources to wholesale purchase, have great storage capacity, and bargaining power. Therefore, they can negotiate flour prices for all their stores throughout all of the regions and define their prices to have less variability with in regions (31).

Inter-Regional Tortillería Cointegration

It was found that *tortillería* prices in the peripheral regions of Mexico presented evidence of cointegration with each other while the central regions did not present significant evidence of such behavior. The lack of cointegration between central and peripheral regions is an interesting finding that could be attributed to the radial transportation system and storage re-distribution activities in Mexico, which is centered in the Central region (22, 29).

When long-term elasticities are analyzed, it was found that all coefficients of the inter-region *tortillería* pairs are near to one. Among them, the North Western-North Eastern pair has the shortest average distance (1091 km), the largest β_1 (0.92) and the lowest α_1 (-0.18) of the three relations.

Further confirming the assumption that distance has a negative effect on price transmission.

When Southern-North Eastern and Southern-North Western price pairs are compared, it was found that long term elasticities and short error correction terms are larger for the first one than for the second. Following to Mc Michel (2009), net production and consumption among these regions could be a factor in the determination of these parameters. When terms of production are compared, North Eastern regions a net consumer of white maize and 90% of their production is concentrated in the Spring-Summer cycle.

The Southern region is slightly a net importer and it has cyclical maize production with deficits and surpluses and produces nearly 80% of its maize in the spring-summer cycle. The North Western region is the biggest net exporter of maize in Mexico and accounts for about 80% of the autumn-winter production (9).

Considering these seasonalities, since the North Western region is the only net producer among them, the likelihood of trade is definitely greater between the North Western and the Southern regions. Therefore, it is clear that larger trade flows can be accounting for increasing levels of price transmission and faster adjustments toward equilibrium from net producing to net consuming countries.

Despite their higher prices and looking at tortilla place of purchase, the *tortillerías* of Central region could be able to retain their market share (76%). This could be explained by an inelastic consumer behavior and greater competitiveness of *tortillerías* in these regions that were previously confirmed by Retes-Matilla and Reyes (2010).

Intra-Regional *Tortillería*-Supermarket Cointegration

It was found that Central region presented the highest long-term elasticity ($\beta_1=0.50$) and the fastest error correction term ($\alpha_1=-0.11$) when compared with the Occidental region, which present a long-term elasticity of 0.49 and an error correction term of -0.08.

The fact that elasticities differ significantly from 1 could be attributed to the higher costs of production (30% higher than supermarkets) and quality differences of *tortillerías* for which certain consumers are willing to pay a higher price (10). Nevertheless, these regions are the only presenting evidence of long-term price cointegration between supermarkets and *tortillerías*.

As demonstrated by Espejel-García (2012), due to they low prices, they are the only two regions where *tortillerías* have been able to retain their larger market share. It can be attributed to the greater importance placed on the price of tortilla by the low and middle-income households.

From this results, infers that the most affected by tortilla's high price are the lower income consumers, who have neglected their quality preference of the nixtamal tortilla by giving greater importance to the price and quantity in order acquire sufficient tortillas to meet their basic dietary needs. In this sense, since consumer preferences have shifted low price as a priority in order to meet basic consumption needs, in seeking for the lowest tortilla prices, they have shifted their place of purchase preference for the cheaper channels, which are: supermarkets, corner stores, and street vendors (15).

Overall, it is very likely that costs structure is much higher for *tortillerías* in regions where supermarket and *tortillería* prices are not cointegrated. The extra cost is likely to emanate from higher transportation costs, which result in higher maize prices. Therefore, infers that *tortillerías* in peripheral regions are not able to react to lower prices of supermarkets, as their market share has eroded.

In contrast, *tortillerías* located in the Central region, which are cointegrated with supermarkets, have maintained their market share due to the combination of greater price transmission and an inelastic demand for tortilla elaborated at *tortillerías*.

These findings appear to validate that radial transportation system in Mexico has benefited the central regions at the expense of the periphery regions, enabling *tortillerías* in Central regions to remain competitive with supermarkets and retain market share. Given that commodities like maize have relative low prices, they are very sensitive to transportation, warehousing and transfer cost. Therefore, it is reasonable to believe that white maize costs could be up to double the price in the extreme areas of Mexico.

From this results, infers that the price of maize paid to producers is not directly related to the big increases in tortilla prices or tortilla price high variability. Instead it attributes their price formation to distribution costs and intermediaries. From a policy perspective, the Mexican government must revise the subsidy policies that primarily benefit grain commercializing companies and large firms. The lack of competition in storage and distribution of maize has adversely affected the end consumer, *tortillerías* located in the periphery, and nixtamal mills located in the periphery.

The World Bank (2007) recommend that the government allow the banking sector to provide better financial mechanisms to maize producers, like allowing them to use their inventories as collateral to acquire loans. This would empower producers and would give them a greater flexibility in commercializing their product, potentially benefiting the nearest *tortilla* consumer markets.

Furthermore, subsidies to *tortillerías* should not encourage new players in an already saturated market, but rather they should encourage the efficient production and the proper economy of scales required to produce *tortillas* at a competitive price. Additionally, FIRA (2014) recommends that *tortillerías* use marketing to promote consumption of their better quality tortillas, so that they are able to maintain market share even when their price is above the supermarket price.

Nevertheless, a lower the cost structure for *tortillerías* would be needed in order to retain the *tortillería* market share necessary for the *tortillerías* currently in business. Achieving proper production economies of scale and direct linkages between maize producers and *tortillerías* is essential to reduction of costs required to maintain their competitiveness.

CONCLUSIONS

The aim of this research was to determine the spatial price transmission level of the Mexican maize tortilla market across different market chains and regions. The results were heterogeneous, demonstrating some evidence of price cointegration between regions for supermarket and *tortillería* prices and within regions between the places of purchase.

The findings of this paper were than viewed in light of the ENIGH survey results, which demonstrated changes in the place of purchase of tortillas between the years 2006 (prior to the crisis) and 2014 (after the crisis). These surveys reveal a notice able reduction in consumption at the *tortillería* channel and an increasing consumption at supermarket or resellers of supermarket tortilla, like corner stores or street vendors. The regions with the greatest consumer reaction seem to be the regions with the lower levels of price transmission between the different market chains.

The *tortillerías* of Central and Occidental regions enjoy some of the lowest tortilla prices in Mexico, which can be

attributed to the cheaper logistic costs of such regions. These regions have a strong supermarket cointegration between them and unlike the other three regions; these are the only two that have evidence of long-term cointegration between supermarket price and *tortillería* price. Accordingly, it is concluded that maize producers and tortilla producers would benefit from a direct transaction through the reduction of commercialization mark up of maize.

Further research is recommended to understand the causes of the regional variability. For example, a technique of dimensionality reduction could be performed, to measure the impact of factors such as insecurity, fuel prices and storage capacity in the spatial price transmission of tortilla.

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Regional reputation as the price premium: estimation of a hedonic model for the wines of Castile-La Mancha

La reputación regional como el diferencial de precio: estimación de un modelo hedónico de precios para los vinos de Castilla-La Mancha

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ABSTRACT

Wine is a multi-attribute product and one of great differentiation. Consumers do not know wines' intrinsic properties before its purchase; consumers need and look for extrinsic signals that allow them to infer those intrinsic properties. To evaluate those intrinsic wine properties, the article uses price as an extrinsic signal to express their value. The price used is a comparison between the prices suggested by the specialized guides and the ones proposed directly to the consumer at the on-line stores. With the hedonic price methodology, which relates price and attributes, the article shows the convergence between the value referenced (price) by the Spanish experts and the one paid (price) in the Spanish market, the former being independent of the interests of the economic agents and the latter not. Regional reputation is the only attribute that positively impacts the price of Castilian wine and is prioritized in the ranking of attributes.

Keywords

hedonic model • wine of Castile • denomination of origin • attributes • experts • on-line sales • Spanish wine market • price premium

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RESUMEN

El vino es un producto de atributos múltiples y de gran diferenciación. Los consumidores no conocen las propiedades intrínsecas de los vinos antes de su compra; los consumidores necesitan y buscan señales extrínsecas que les permitan inferir esas propiedades intrínsecas. Para evaluar esas propiedades intrínsecas del vino, este artículo usa el precio como una señal extrínseca para expresar su valor. El precio utilizado es una comparación entre los precios sugeridos por las guías especializadas y los que se proponen directamente al consumidor en las tiendas en línea. Con la metodología de precios hedónicos, que relaciona precios y atributos, este artículo muestra la convergencia entre el valor referenciado (precio) por los expertos españoles y el valor pagado (precio) en el mercado español, siendo el primero independiente de los intereses de los agentes económicos y el último no. La reputación regional es el único atributo que impacta positivamente en el precio del vino castellano y se prioriza en el ranking de atributos al momento de compra.

Palabras clave

modelo hedónico de precios • vino de Castilla • denominación de origen • atributos • expertos • ventas en línea • mercado del vino español • diferencial de precio

INTRODUCTION

The wine region of Castile-La Mancha (Spain) has the largest vineyard in the world, with an area of 580,000 hectares, representing 51% of Spain's vineyard, 17.6% of the European vineyard, and 7.4% of the world's vineyard (29). Its estimated production is, on average, usually more than 50% of the total of Spain, approximately 10% of the European Union-27, and 7% of the world (29). For comparative purposes, Castile-La Mancha produces the same amount of wine in hectoliters as the fourth world producer, the USA, 80% of what is produced in Australia or Argentina, and more than double of what is produced in Chile (29).

Castile-La Mancha has 13 wines within the category of Quality Wines Produced in Selected Regions, which represents 15% of the Spanish total production. 70% of the categories have acquired a level of protection of

"Denomination of Origin" (Protected Designation of Origin), and those are: Almansa, Jumilla, La Mancha, Manchuela, Méntrida, Mondejar, Ribera del Júcar, Uclés, Valdepeñas. The remaining 30% of the categories have acquired a level of protection called Wine of the Land: Dehesa del Carrizal, Domain of Valdepusa, Finca Élez and Guijoso. Of all the "Denomination of Origin" (Protected Designation of Origin) in Spain, La Mancha is the largest surface with 187,000 ha, which is 29.33% of the total national surface for quality wines (29).

Wine in Castile-La Mancha is economically relevant; it is also an important part of its traditions, and its society. Over the last two decades in Castile-La Mancha and Spain, wineries have worked hard to market their wines appealing to their origin, creating certain entities of differentiation with links to land, and

its geographical origin. Therefore, it is necessary to know what qualities of the wine of Castile-La Mancha, how and to what extent they condition their price, as well as their competitiveness and their acceptance in the markets.

Determining which aspects condition the price of a product is not a simple task. Wine companies continually develop differentiation strategies, generating a large number of products with varying combinations of attributes which affect the final price. However, the intrinsic attributes of a product are not traded directly in the market and only present value within the product that contains them. Obviously, when a consumer acquires those attributes he must pay an amount to the seller, the question being whether what is paid by the customer is close to the qualities of the wine.

This article is to compare whether the market price of a particular bottle of wine from the Spanish region of Castile-La Mancha converges with the value of its qualities, measuring these qualities using the prices presented in a reputed Spanish specialized guide and in several Spanish on-line stores as proxies. Research focused on wine products and markets are multifaceted and intricate, and for this reason it is difficult to ignore the role of context; as mentioned already the geographical context of this research is limited to Castilian wines being sold in the Spanish market.

Twenty on-line Spanish stores were chosen for this research, and the famous and well-known specialized guide Peñín (2010) was also selected: Alimentos de Cuenca, Catavinum, Cervezas y vinos, Elviwines, Enoteca Barolo, Intervinos, La Tienda de los Vinos, Lavinia, Pasión Vinícola, Quijote Vinos, Sibaritia, Torrevinos, Vegaval, Verema, Vinissimus, Vino Gusto, Vinos Checa, Vino Selección, Vinos en Casa, Vitivinos.

A consequent objective of this article is to study whether the difference in the prices of Castilian wine are associated with factors of quality and/or economic nature. Being that wine a highly differentiated product, the hedonic price model suits perfectly and allows the identification of attributes having the biggest impact in consumers' willingness to pay.

Different characteristics that make up a product, in this case Castilian wine, are reflected in its market price. It is therefore assumed that its price can be decomposed according to its attributes and, once the hedonic price model has been estimated, it would be possible to assign an implicit price premium to each of these characteristics.

For the case of wine, objectively measured attributes considered in hedonic price models are, to mention a few, grape variety, vintage, alcohol content, other technical quality attributes, the landscape or reputation of a particular wine region such as Castile-La Mancha, Bordeaux, or Mendocino and the jury grade received by a wine (11). Hedonic pricing theory, which is well known in the field of economics, has broadly been used to scrutinize and examine prices and consumer preferences for numerous products including wine (14, 25, 26, 27, 28, 38, 39).

The article is structured as follows. section one presents the literature review. section two describes the database and the method, and the results are discussed. Finally, the conclusions and references are presented.

Literature review

The basic idea of the hedonic valuation methodology is that in the price of some goods, the price of each one of its attributes is implicit. These goods give utility to the consumers based on the characteristics that compose them. But these features are not traded separately but transferred into

a package when you buy a good. Different studies point to Court (1941) as the first investigator to coin the term hedonic model, although the origin of its applications is more controversial. There is no doubt that the theory of hedonic models has been shaped over time, Lancaster (1966) developed the fundamentals to estimate the value of utility generated by the characteristics of a good, and Rosen (1974) established the theoretical support of the hedonic price model.

Hedonic pricing has broadly been used in economics and marketing literature as a theoretical model to examine consumer preferences for different product attributes. Lancaster (1966) presented the idea that consumers value attributes of products rather than whole products. This idea has been prolonged by consumer researchers in various ways contributing to a large number of theories and has been made accessible to practitioners and the public (*e.g.* Kotler and Keller (2009)). In a comparable stream of literature, and as previously mentioned, economists also built on Lancaster's (1966) ideas along with others in the development of hedonic pricing theory, which was formally posed by Rosen (1974).

Hedonic pricing theory describes the aggregate preferences consumers have for product attributes in a particular market. This is done by examining the relationship between these attributes and prices. Attributes which attract price premiums are believed to be valuable to consumers while those which attract lower prices are considered as less attractive.

Early studies which used hedonic pricing theory to explain consumer standards associated to wine focused on the role of various types of attributes on wine prices (2, 6, 8, 14, 21, 25, 26, 40). Oczkowski (2001) summarized these attributes into four key categories: chemical, climatic, sensory, and objective.

"Chemical attributes are measurable properties of the wine relating to its chemical composition such as sugar and acidity levels" (Oczkowski, 2001). "Climatic attributes relate to the weather in the time and place where the grapes were grown; by influencing grape quality and characteristics, these can have an influence on the taste and quality of wine" (Oczkowski, 2001).

However, "chemical and climatic attributes have been deemed unsuitable for inclusion in hedonic pricing studies because consumers are usually unable to access or understand this type of information when making wine purchase decisions" (Oczkowski, 2001). Further, "their influence on wine quality and taste is often poorly understood beyond wine makers, hence sensory attributes are also unlikely to affect the price consumers pay" (Oczkowski, 2001).

Objective attributes are those which can easily be obtained by consumers, by reading the information included on the wine bottle labels, such as: grape variety, vintage year, and wine region (27, 37). Aggarwal (2004) referred to these attributes not as 'objective', but as 'objectively measured'. This is because consumers often build complicated and subjective relationships with attributes such as brands or regions (1), and hence they are not always considered to be objective within consumer behavior and marketing literature (1). Previous research has established the importance of a number of objectively measured wine attributes, which may have a relationship with the price consumers pay for wine. These attributes, such as grape variety, vintage or wine age, and region of origin will be the main focus of this article and will be considered individually below.

Grape variety

The relationship between grape varieties and price has been considered previously in the hedonic pricing literature (2, 4, 26, 35, 36, 39). These studies have noted that "grape varieties which are fashionable at the time of the study tend to attract higher prices than those which are old fashioned or out of trend" (26).

Vintage

Numerous hedonic pricing studies have considered the influence of either vintage or age on the price of wines (7, 26, 36, 37, 39). These studies have largely noted that older wines tend to attract higher prices. This is likely because "consumers value the prestige of wines" (22), which has already aged and are ready to drink. Alternatively Steiner (2004a) noted that "the higher prices may also reflect storage costs which are worn by retailers and saved by consumers in the case of purchasing older vintages".

Region

"Region of origin covers aspects of the reputation and brand of the geographical area where the wine is produced" (Easingwood *et al.*, 2011). It is a different idea from "terroir" which relates to the impact of the physical features such as soil conditions on the grapes (10). "Marketing wine by region allows for the development of a 'regional brand' which can create a sustained competitive advantage for all producers within that region" (12). Consumer focused studies in numerous contexts have found region to be a significant factor for many consumers (17, 37) and "its absence from a wine label may lead to negative perceptions of quality" (16). In addition, numerous hedonic pricing studies have examined the influence regional reputations have on wine prices (4, 35, 38, 39). Schamel and Anderson (2003) and Bicknell *et al.* (2005) suggest that region of origin

does affect the price that consumers pay for wine. Hence, for place of origin or region, a strong consensus (23, 34, 38) has risen over the fact that the more specific the labelling of the place of origin, the higher the price. "Moreover, a positive trend has been distinguished toward more regional differentiation" (Estrella *et al.*, 2012).

Prices

For recommended prices to use in the hedonic pricing studies, two different sources have been used through literature: specialized guide prices and retailer prices. The most widely used source has been prices from wine guides or wine publications (11). This choice has been generally explained by the data's accessibility to the wine consuming public at large. Moreover, as sustained by Ortuzar-Gana and Alfranca-Burriel (2010) these recommended prices could be useful because they do not take into account the seasonal discounts and are independent of the retailer characteristics (11). Even if they are widely used, wine guides prices have been considered inappropriate for estimating hedonic price equations by many authors (5, 8, 15, 41). Because of these limitations in the use of prices from only specialized guides, and as previously showed, the present article uses both, specialized guide prices and retailer prices.

Data and methods

Sample and variables

From the famous Peñín Guide (2010) the characteristics and price of the wines presented were accounted. From the on-line distribution points, only price was accounted. At first, all the red, white and rosé wines of the guide were chosen, 331 in total, but these were reduced to 183 because not all the bottles were found at the on-line sales outlets. Below, the

study present the main characteristics of each variable.

The price of the product is the endogenous variable of this study; it captures the effect on the valuation of the different attributes of the wine. Price in the sample varies between 1.9 € and 125 € in reds, between € 1,5 and € 13,95 in whites, and between € 1.3 and € 8.8 in rosés. From the difference between the minimum and the maximum, one could understand that, especially in red wine, there are attributes that cause important differences in price.

The exogenous variables are the considered attributes $Q_{j,i}$: the "Denomination of Origin" (Protected Designation of Origin), the quality, the grape variety, the time of fermentation, and the year of harvest or vintage. They are quantified as binary variables, taking the value "1" when the mentioned characteristic occurs and "0" if it does not occur.

The "Denomination of Origin" (Protected Designation of Origin), $Q_{DO,i}$, is a reference to the provenance of the wine. The different denominations of origin present in the sample are Manchuela, La Mancha, Valdepeñas, Ribera del Júcar, Jumilla, Méntrida and Almansa.

To analyze the *quality* attribute, $Q_{QUAL,i}$, the ranking of the publication Peñín Guide (2010) was used. The guide qualifies wines in exceptional, excellent, very good, and acceptable. Only 2% of the Guide wines obtained the qualification of exceptional.

The different types of grape varieties, $Q_{VA,i}$, generate a high diversity of products, a wide variety of flavors and combinations. The grape varieties classified for this study, depending on the type of wine, are Cabernet Sauvignon, Bobal, Cencibel, Merlot, Petit

Verdot, Syrah, Tempranillo, Monastrel, Garnacha, Garnacha Tinta, Sauvignon Blanc Verdejo Viura, Airen Macabeo, Moscatel, and Chardonnay. Although many wines are produced with different grape varieties, it was given a value of "1" to the predominant in its composition.

Depending on the duration of the *fermentation* of the wine, $Q_{FER,i}$, different types of wine will be obtained. Their "technical files" will present very personal characteristics that will classify them according to their grape of production, their aroma, their flavors, and their maturation, in wines that are Young, Crianza, Reserve, Grand Reserve and Fermented in Barrel.

The *vintage* of the wines, $Q_{VINT,i}$ is the year of the harvest of the grapes, and it goes in this study from 1998 to 2008.

Functional form of the model

To develop the study the theory of hedonic models was used, $P_i = f(Q_{j,i}, \mathcal{E}_i)$. It considers that the wine price (P_i) is implicitly present in each of its attributes ($Q_{j,i}$). Considering the Box-Cox technique for the identification of the functional form (f) and that the exogenous variables are binary numbers, it is obtained ¹:

where:

\mathcal{E}_i = the random disturbance that follows a normal distribution of zero mean and constant variance $\mathcal{E}_i \approx N(0, \sigma_{\mathcal{E}}^2 I_n)$

The variable P_i assumes the values of a) red wine, b) white wine, and c) rosé wine. Two sub-models of model (1) were estimated; both sub-models determine the valuation of price, one by experts and the other by distributors.

$$1 \quad P_i^\lambda = \beta_0 + \beta_1 \sum_{DO=1}^g Q_{DO,i} + \beta_2 \sum_{QUAL=1}^h Q_{QUAL,i} + \beta_3 \sum_{VA=1}^k Q_{VA,i} + \beta_4 \sum_{FER=1}^l Q_{FER,i} + \beta_5 \sum_{VINT=1}^m Q_{VINT,i} + \mathcal{E}_i \quad (1)$$

The SPSS-15 econometric software has been used to obtain the statistical and econometric results.

Descriptive analysis

The general analysis of the origin of the price of wine allows us to draw some conclusions in order to put in context their economic situation.

In table 1 the descriptive statistics of the endogenous variables, *P*, are shown, while in table 2 (page 300-301), the exogenous variables frequencies are presented.

As can be seen in table 1, there is a range of variation of results between the prices on the specialized guide and the prices at the on-line stores that is confirmed with the non-parametric contrast of Mann-Whitney, with a p-value less than 0,05 (table 3, page 302).

A non-parametric contrast was chosen for the rejection of the Null Normality Hypothesis of the endogenous variable *P* in the Kolmogorov-Smirnov test, also with p-value less than 0.05 (table 3, page 302).

This result could be explained following Bello and Cervantes (2002), they indicate that the quality of a wine is inferred by its

value and quality, in short, if the price of a wine is more expensive is because it is better. Then, it may also be argued that if the on-line stores prices are higher than the ones in the specialized guide that is a consequence of the suppliers valuing the qualities of the wine more than the experts.

However Ruíz *et al.* (2004) mentioned that "from the perspective of companies, their knowledge of the market can lead them to develop opportunistic behavior if it gives them higher levels of profitability". Companies or on-line stores may be interested in creating commercial communication mechanisms, under an apparent fairness environment, that will interest consumers in making their purchasing decisions based on non-impartial advice, issued as independent advice.

On the other hand, specialized guides summarize the information about the product, providing rankings in an independent way that helps the consumer to evaluate the information and make the decision most appropriate to their wants and needs.

Table 1. Endogenous variables descriptive statistics.

Tabla 1. Estadísticas descriptivas de las variables endógenas.

	N	Minimum	Maximum	Mean	Typical Deviation
Red Wine					
Specialized Guide P	115	1.9	113.0	10.893	15.9643
On-line P	115	1.20	125.00	11.8467	17.58990
White Wine					
Specialized Guide P	47	1.5	11.0	3.541	2.2343
On-line P	47	1.72	13.95	4.3211	2.75568
Rosé Wine					
Specialized Guide P	21	1.3	4.0	2.633	0.7370
On-line P	21	1.72	8.80	3.3386	1.46296

Table 2. Exogenous variables frequencies.
Tabla 2. Frecuencias de las variables exógenas.

			Frecuency (value 1)	Frecuency (value 0)	N
Red	DO	DO Manchuela	12	103	115
		DO La Mancha	30	85	115
		DO Valdepeñas	11	104	115
		DO Ribera Júcar	10	105	115
		DO Jumilla	38	77	115
		DO Métrida	10	105	115
		DO Almansa	4	111	115
	Quality	Exceptional	4	111	115
		Excellent	28	87	115
		Very Good	78	37	115
		Acceptable	5	110	115
	Grape Variety	Cabernet-Sauvignon	12	103	115
		Bobal	8	107	115
		Merlot	5	110	115
		Petit Verdot	5	110	115
		Syrah	20	95	115
		Tempranillo	37	78	115
		Monastrell	26	89	115
		Garnacha	2	113	115
		Garnacha tinta	7	108	115
		Fermentation	Young	81	34
	Crianza		18	97	115
	Reserve		11	104	115
	G.Reserve		4	111	115
		Fermented in Barrel	1	114	115
	Vintage	1998	1	114	115
		1999	1	114	115
		2000	1	114	115
		2001	2	113	115
		2002	4	111	115
		2003	7	108	115
		2004	6	109	115
		2005	17	98	115
		2006	24	91	115
	2007	32	83	115	
	2008	20	95	115	

Note: N are the total observations. A variable has a value of "1" if the wine has that attribute and "0" otherwise.
 Nota: N son las observaciones totales. Una variable tiene un valor de "1" si el vino tiene ese atributo y de "0" en caso contrario.

Table 2 (cont). Exogenous variables frequencies.
Tabla 2 (cont). Frecuencias de las variables exógenas.

			Frequency (value 1)	Frequency (value 0)	N	
White	DO	DO Manchuela	7	40	47	
		DO La Mancha	24	23	47	
		DO Valdepeñas	6	41	47	
		DO Ribera Júcar	2	45	47	
		DO Jumilla	6	41	47	
		DO Almansa	2	45	47	
	Quality	Very Good	40	7	47	
		Acceptable	7	40	47	
	Grape Variety	Sauvignon -blanc	7	40	47	
		Verdejo	10	37	47	
		Viura	1	46	47	
		Airen	12	35	47	
		Macabeo	16	31	47	
		Moscatel	3	44	47	
		Chardonay	2	45	47	
		Fermentation	Young	44	3	47
			Fermented in Barrel	3	44	47
		Vintage	2004	1	46	47
	2007		7	40	47	
	2008		39	8	47	
Rosé	DO	DO Manchuela	4	17	21	
		DO La Mancha	10	11	21	
		DO Valdepeñas	2	19	21	
		DO Jumilla	5	16	21	
	Quality	Very Good	20	1	21	
		Acceptable	1	20	21	
	Grape Variety	Cabernet-Sauvignon	20	1	21	
		Bobal	4	17	21	
		Tempranillo	9	12	21	
		Monastrell	5	16	21	
		Garnacha tinta	2	19	21	
Fermentation	Young	21	0	21		
Vintage	2008	21	0	21		

Note: N are the total observations. A variable has a value of "1" if the wine has that attribute and "0" otherwise.
 Nota: N son las observaciones totales. Una variable tiene un valor de "1" si el vino tiene ese atributo y de "0" en caso contrario.

Table 3. Contrasts of Kolmogorov-Smirnov and Mann-Whitney.
Tabla 3. Contrastes de Kolmogorov-Smirnov y Mann-Whitney.

	Kolmogorov-Smirnov		Mann-Whitney	
	Statistic	Sig.	Statistic	Sig.
Red Wine	0.282	0.000	6112.500	0.032
White Wine	0.266	0.000	801.500	0.002
Rosé Wine	0.151	0.017	148.500	0.063

RESULTS

To identify the lambda value of P_i , the contrasts of Ramsey, White, and Durbin Watson (table 4, page 303) were applied.

As a result, the logarithmic function is the chosen one for the red wine, the reciprocal for the white wine, and the double logarithmic function for the rosé wine. Their analytical expressions are ²:

Because they are complex models, as they incorporate many effects that can influence prices, the Backward estimation method was applied. Following this method, the less influential variable is eliminated at each stage, until no more terms are possible to delete. The estimation of the corresponding models is shown in table 5 (page 304).

The contrasts of joint significance F-Snedecor, with a p-value less than 0.05,

and the relatively high goodness of fit, measured by the coefficient of determination R^2 , and a sum of residual squares SCR close to zero indicate a valid estimate (table 6, page 304).

Once the estimate is accepted, the results needs interpretation. To facilitate the interpretation of the fictitious variables, it is convenient to calculate the relative percentage effect of the dummy variable on the price. To do this, the estimate of the percentage impact developed by Kennedy (1981) is used:

$$IP_i = 100 * \left[\exp(\hat{\beta}_i - 0.5Var(\hat{\beta}_i)) - 1 \right]$$

where:
 $\hat{\beta}$ = the estimated coefficient

2

$$\ln P_i (\text{red}) = \beta_0 + \beta_1 \sum_{DO=1}^7 Q_{DO,i} + \beta_2 \sum_{QUAL=1}^4 Q_{QUAL,i} + \beta_3 \sum_{VA=1}^9 Q_{VA,i} + \beta_4 \sum_{FER=1}^4 Q_{FER,i} + \beta_5 \sum_{VINT=1}^{11} Q_{VINT,i} + \varepsilon_i \quad (2)$$

$$1/P_i (\text{white}) = \beta_0 + \beta_1 \sum_{DO=1}^7 Q_{DO,i} + \beta_2 \sum_{QUAL=1}^4 Q_{QUAL,i} + \beta_3 \sum_{VA=1}^9 Q_{VA,i} + \beta_4 \sum_{FER=1}^4 Q_{FER,i} + \beta_5 \sum_{VINT=1}^{11} Q_{VINT,i} + \varepsilon_i \quad (3)$$

$$\ln P_i (\text{rosé}) = \beta_0 + \beta_1 \sum_{DO=1}^7 Q_{DO,i} + \beta_2 \sum_{QUAL=1}^4 Q_{QUAL,i} + \beta_3 \sum_{VA=1}^9 Q_{VA,i} + \beta_4 \sum_{FER=1}^4 Q_{FER,i} + \beta_5 \sum_{VINT=1}^{11} Q_{VINT,i} + \varepsilon_i \quad (4)$$

Table 4. Evaluation test for the functional form of the model.
Tabla 4. Prueba de evaluación de la forma funcional del modelo.

Evaluation Test functional form						
Type of price	Functional form	White		Ramsey Test		Durbin Watson
			p-value	Reset	p-value	
	Linear	60.17	0.0012	84.94	0	2.17 *
Red Wine	Non linear					
Specialized	Logarithmic **	31.77	0.427*	0.028	0.867*	2.18 *
Guide Price	Double logarithmic	28.42	0.599*	4.82	0.028	2.17 *
	Reciprocal	34.59	0.3*	10.79	0	2.12 *
	Square root	45.85	0.04	15.82	0	2.15 *
	Linear	60.41	0	78.51	0	2 *
Red Wine	Non linear					
On-line Price	Logarithmic **	27.57	0.64*	0.57	0.45*	1.96 *
	Double logarithmic	14.56	0.99*	2.75	0.097*	1.87 *
	Reciprocal	17.14	0.97*	6.94	0.0008	1.81 *
	Square root	45.61	0.04	21.55	0	1.96 *
	Linear	25.47	0.043	27.62	0	1.46
White Wine	Non linear					
Specialized	Logarithmic	22.08	0.10*	11.55	0	1.54
Guide Price	Double logarithmic	18.59	0.23*	2.14	0.14*	1.60 *
	Reciprocal **	18.40	0.24*	1.68	0.19*	1.60 *
	Square Root	23.74	0.07*	18.71	0	1.50 *
	Linear	30.87	0.009	26.04	0	1.11
White Wine	Non linear					
On-line Price	Logarithmic	19.28	0.2*	3.16	0.075*	1.27
	Double logarithmic	6.014	0.97*	0.168	0.68*	1.10
	Reciprocal **	6.24	0.975*	0.64	0.42*	1.1
	Square Root	29.4	0.014	11.8	0	1.22
	Linear	7.32	0.29*	8.65	0.0032	1.86 *
Rosé Wine	Non linear					
Specialized	Logarithmic	5.21	0.51*	8.74	0.003	1.95 *
Guide Price	Double logarithmic**	6.012	0.42*	0.081	0.775*	1.81 *
	Reciprocal	5.22	0.51*	4.7	0.03	1.92 *
	Square Root	6.29	0.39*	23.25	0	1.92 *
	Linear	5.5	0.48*	3.928	0.047	2.18 *
Rosé Wine	Non linear					
On-line Price	Logarithmic	5.45	0.49*	8.69	0.003	2.063*
	Double logarithmic**	4.82	0.56*	0.79	0.37*	2.03 *
	Reciprocal	4.74	0.57*	4.25	0.039	2.045*
	Square Root	5.54	0.47*	14.32	0	2.11 *

* Significant at 5% significance. ** Selected forms according to the aforementioned tests.

* Significativo al 5% de importancia. ** Fórmulas seleccionadas de acuerdo con las pruebas mencionadas.

Table 5. Backward estimations of the hedonic models.
Tabla 5. Estimaciones hacia atrás de los modelos hedónicos.

			Guide Coefficient	Typical Error	Online Sotore Coefficient	Typical Error
Red	DO	DO Manchuela	0.190	0.040	0.149	0.039
		DO La Mancha	0.127	0.025	0.152	0.026
		DO Valdepeñas	0.115	0.032	0.108	0.034
		DO Ribera Júcar	0.117	0.034	0.115	0.035
	Quality	Exceptional	-0.262	0.0460	-0.215	0.048
		Excellent	-0.132	0.023	-0.115	0.024
		Acceptable	0.095	0.042	0.094	0.044
	Grape Variety	Cabernet-Sauvignon	-0.072	0.027	-0.070	0.028
		Bobal	-0.196	0.048	-0.138	0.047
		Merlot	-0.096	0.040	-0.085	0.042
		Petit Verdot	-0.163	0.039	-0.145	0.040
	Fermentation	Syrah	-0.043	0.024	-	-
		Young	-0.211	0.031	-0.211	0.031
		Crianza	-0.213	0.032	-0.205	0.040
Reserve		-0.300	0.044	-0.301	0.040	
Vintage	G.Reserve	-0.394	0.047	-0.382	0.045	
	2004	-0.090	0.037	-0.073	0.036	
	2005	-0.057	0.025	-0.037	0.024	
	2008	0.052	0.024	0.068	0.026	
White	DO	DO Manchuela	-	-	-0.151	0.032
		DO Almansa	-0.253	0.080	-0.067	0.055
		DO Jumilla	-0.107	0.048	-0.058	0.033
		DO Valdepeñas	-0.085	0.048	0.099	0.034
	Grape Variety	Chardonnay	0.051	0.077	-0.094	0.054
		Moscatel	0.018	0.064	0.014	0.044
		Sauvignon Blanc	-0.338	0.046	-0.040	0.032
	Verdejo	-0.025	0.041	0.115	0.028	
Rosé	DO	DO Manchuela	-0.349	0.118	-0.363	0.164
		DO Jumilla	0.286	0.108	0.302	0.151

Table 6. F-Snedecor, R² and SCR. / F-Snedecor, R² y SCR.

	F-Snedecor	R ²	Scr
Red	14.554 (0.000)	0.855	0.652
	13.012 (0.000)	0.825	0.744
White	10.685(0.000)	0.742	0.195
	9.872 (0.000)	0.832	0.186
Rosé	10.799 (0.001)	0.739	0.747
	6.109 (0.009)	0.711	1.445

In parentheses the p-value associated with the Snedecor F.
 Entre paréntesis, el p-valor asociado con el Snedecor F.

$Var(\hat{\beta})$ is its variance (table 4, page 303, values and $Var(\hat{\beta})=(\text{typical error})^2$). The impacts are presented in table 7 (page 306).

In general, the assessment, both positive and negative, is stronger in the guides of the experts than in the online stores assessments. From the results of the red wine, it should be noted that both the experts on the guides and the on-line vendors consider the Denomination of Origin (Protected Designation of Origin) the only attribute that, on average, would increase the price of red wine of Castile-La Mancha (about 14%).

The DO most valued by all is the DO La Manchuela. Both the experts on the guides and the on-line vendors agree that the impact on the price of other attributes is negative. The results shown regarding grape variety may happen because these are grape varieties considered to be traditional and as noted in Oczkowski (1994), Angulo *et al.* (2000), Schamel and Anderson (2003), Steiner (2004b), Bicknell *et al.* (2005), and Schamel (2006), the grape varieties that are fashionable at the time of the study tend to attract higher prices than those that are outdated or are out of trend. As for the vintage, the impact is also negative since they are very recent at the date of price assessment (year 2010), and as noted in Lee and Zhao (2013), older wines tend to attract higher prices.

As for white wines it should be mentioned that, on average, of all the significant attributes, none have a positive impact on price. However, if we analyze the results at the individual level, the Moscatel grape variety is the only one with a positive impact.

Finally, and regarding rosé wines, on average, the impact the Denomination of

Origin (Protected Designation of Origin) has is negative, except for the DO La Manchuela that, at the individual level, has a positive impact. Here, contrary to what happened with red and white wines, the positive and negative evaluations have greater weight at the points of sale (on-line stores).

The only attribute positively valued by both parties, specialized guides and on-line stores, is the Denomination of Origin (Protected Designation of Origin) and if a ranking of recommendations of attributes was to be done, it would only agree on this characteristic. This reflects that this market values more wines originated in a specific geographic area. It may imply that positioning a specific wine geographic area may justify a difference in the price of the wine with respect to those wines originated in global zones where no geographical area is shown.

The article confirmed that a wine attribute such as region of origin can attract price premiums; consumers attach considerable importance to the perceived origin of a labeled region. The relationships between variety and price, and age and price were less clear. Nevertheless, the results suggest that a relationship does exist.

The present article also focuses on defining if the determinants of the price of Castile-La Mancha wine are equally appreciated by the specialized guides and the on-line sales outlets. In principle, specialized guides experts are professionals who show their knowledge and criteria with no external influence. On-line sales outlets deal directly towards a very specialized end consumer, generally interested and very well informed.

Table 7. Impact measurement.
Tabla 7. Medida de impacto

		Impact guide	Impact online store	Difference	Average impacts guide	Average impacts online store		
Red	DO	DO Manchuela	20.80	15.95	4.85	+15%	+1.4%	
		DO La Mancha	13.52	16.29	-2.76			
		DO Valdepeñas	12.10	11.30	0.80			
		DO Ribera Júcar	12.36	12.09	0.26			
		Exceptional	-23.15	-19.42	-3.72	-9%	-7%	
		Excellent	-12.41	-10.86	-1.55			
		Acceptable	9.91	9.74	0.17			
		Grape Variety	Cabernet-Sauvignon	-7.00	-6.76	-0.24	-11%	-10.4%
			Bobal	-17.92	-13.01	-4.91		
			Merlot	-9.20	-8.28	-0.92		
White			Petit Verdot	-15.13	-13.62	-1.51		
			Syrah	-4.23				
			Young	-19.39	-19.37	-0.02	-24,5%	-24%
			Crianza	-19.54	-18.90	-0.64		
			Reserve	-26.26	-26.34	0.09		
			G.Reserve	-32.92	-32.10	-0.82		
			2004	-8.65	-7.09	-1.56	-3%	-1,26%
			2005	-5.54	-3.66	-1.88		
			2008	5.32	6.96	-1.64		
			DO Manchuela	-23.22	-6.62	-9.07	-14%	-4%
Rosé			DO Jumilla	-10.27	-5.72	-3.66		
			DO Valdepeñas	-8.29	9.67	-2.57		
			Chardonnay	5.04	-9.26	14.30	0%	-6%
			Moscatel	1.65	1.29	0.37		
			Sauvignon Blanc	-29.04	-4.05	-24.99		
			Verdejo	-2.69	12.08	-14.77		
			DO Manchuela	-29.96	-31.35	1.39	-1,2%	-1,2%
			DO Jumilla	32.32	33.68	-1.35		

Note: the third column is the difference between the first column and the second column. The average is the arithmetic mean of the column of the differences of each group of attributes.

Nota: la tercera columna es la diferencia entre la primera columna y la segunda columna. El promedio es la media aritmética de la columna de las diferencias de cada grupo de atributos.

The price valuations are not similar between the specialized guides and the on-line sales outlets, being prices generally higher on-line than those referenced in the specialized guides. However, the correlation between the two series has the same sign indicating that they follow the same standard. In addition, the impacts of each attribute on price also coincide in the sign, but those of the experts in the guides are more intense, both positive and negative; experts in the guides tend to exalt and devalue wines more than what on-line sellers do.

In view of the results shown, it is possible to acknowledge of a quasi-convergence between on-line sales prices and those in specialized guides. If an objective evaluation of wine quality would be carried out, there should be convergence, not unanimity, in the price valuations. The discrepancies detected in these wine prices raise questions about the causes of these differences.

The fundamental explanation of these differences may be found at the economic level because, on the one hand, and as pointed out by Ruiz *et al.* (2004), the guides are not always impartial since there are certain doses of opportunistic behavior by wineries to partially convert these guides into a commercial communication mechanism for them, and, on the other hand, the tendency of the wineries' strategies to use differentiation in a world flooded of brands or regions. Therefore, there is not a total objective mechanism for assessing the quality level of wines, commercial opportunity would be the main cause of that non-convergence between the still close referenced prices in some channels.

CONCLUSIONS

A hedonic price model was calculated for wines from Castile-La Mancha sold in Spain at on-line stores, using the prices presented in these several Spanish on-line stores and the prices presented at a reputed Spanish specialized guide as proxies.

The price of Castilian wine is the endogenous variable of this study; it captures the effect on the valuation of the different attributes of the wine.

The exogenous variables are the considered attributes: the "Denomination of Origin" (Protected Designation of Origin), the quality (following standards from the reputed Peñín Guide, 2010), the grape variety, the time of fermentation, and the year of harvest or vintage.

In an economy of highly differentiated products and complex purchasing decisions, the Hedonic price model offers a valid way to identify quality attributes influencing consumers' marginal willingness to pay and to estimate the implicit price of these attributes. These estimations provide useful information aiming to improve the producers' and intermediates' marketing strategies, which can be fine-tuned according to the different products' characteristics and target consumers (11). As Angulo *et al.* (2000) point out, the basic hedonic hypothesis is that wines are valued for their characteristics and not as the wine itself. The price of a wine can then be analyzed as the sum of the prices of the attributes that define this wine, and not the wine as a whole. Ferro and Benito-Amaro (2017) allocate monetary values to the underlying characteristics included in a good; these characteristics can reveal a lot about the attributes that consumers value in wine.

In this article, two main conclusions are identified. The first is that the assessment of the experts, regardless of the origin and the attributes valued in the wine, coincide, and must be taken into account. This corroborates what was indicated in Angulo *et al.* (2000). The second is that the variable "Denomination of Origin" (Protected Designation of Origin) is the only one that has a significant and positive impact on the price of the wine of Castile.

Some other studies using hedonic techniques have reached similar conclusions for Spanish wines. Angulo *et al.* (2000) proposed a function of Hedonic prices for red wines, the results showed that the variables "Denomination of Origin" (Protected Designation of Origin) and harvest year or vintage were the main determinants of the market price. In this case, the harvest year or vintage is not decisive since the year of reference was almost simultaneous with respect to the

valuation of the guide and of the prices at the on-line stores. Morilla and Martínez (2002), in the same line, concluded that the quality assessment has a high impact on the price.

Therefore, and particularly in Spain and Castile, wine marketing strategies should deepen in systematizing the territorial differentiation of origin or its protected geography. In such a segmented market as it is wine selling, it is necessary to simplify the parameters for the consumer and provide wine differentiation and diversification basically by origin, terroir and/or spatial determinants.

As proved, the take-off of online sales is highly conditioned by the opinion of guides and experts. It is so recommendable that the strategy in the expansion of this commercial path is carried out in a way that the comments expressed by these experts and opinion formers is given the particular relevance they have.

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An application of network theory to territorial analysis: The case of Yaqui Valley networks (2006 and 2016)

Una aplicación de la teoría de redes al análisis territorial: El caso de las redes del Valle del Yaqui (2006 y 2016)

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ABSTRACT

The Yaqui Valley territory in the northwest of Mexico was analyzed using networks theory that determines the flows density and actors centrality among the agricultural sector. These networks were those of public, financial, commercial, technical, and social policy management. The current research has been carried out with the general objective of identifying the actors that make up the networks in the Yaqui territory and their change in densities as one of the factors that determine the effectiveness of the policy applied in the territory, comparing two samples of 2006 and 2016. Linked to this objective, it is proposed to describe the obstacles for the densification of networks that favor the agricultural producers labor in the Yaqui Valley territory. The obtained results indicate a clear disarticulation in the different networks of study that can elicit serious social problems of inequality among the actors that are active in the agricultural sector. The networks found in the territory of the Yaqui Valley have been disarticulated over time, because ten years after being analyzed for the first time, the calculation of densities has been reduced by 24% on average, and the truss of the social sector practically tends to disappear.

Keywords

networks • effectiveness of public policies • inequality • territory • Yaqui Valley

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RESUMEN

Se analizó el territorio del Valle del Yaqui, en el noroeste de México, utilizando la teoría de redes que permiten determinar la densidad de flujos y centralidad de actores del sector agrícola. Estas redes fueron las de gestión de política pública, financiera, comercial, técnica y social. La presente investigación se ha llevado a cabo con el objetivo general de identificar los actores que conforman las redes en el territorio Yaqui y su cambio en las densidades como uno de los factores que determinan la eficacia de la política aplicada en el territorio, comparando dos muestras de 2006 y 2016. Vinculado a este objetivo se plantea describir los obstáculos para la densificación de las redes que favorezcan el trabajo de los productores agrícolas en el territorio del valle del Yaqui. Los resultados obtenidos indican una clara desarticulación en las diferentes redes, que puede provocar serios problemas sociales de desigualdad entre los actores que se encuentran activos en el sector agrícola. Las redes encontradas en el territorio del Valle del Yaqui, han ido desarticulándose a través del tiempo, debido a que, a diez años de haberse analizado por primera vez, el cálculo de las densidades se ha reducido en un 24% en promedio, y el entramado del sector social prácticamente tiende a desaparecer.

Palabras clave

redes • eficacia de las políticas públicas • desigualdad • territorio • Valle del Yaqui

INTRODUCTION

The relationship among people, societies, cultures, companies, and institutions allows opportunities for growth in labor, production, politics, and economics; that leads to the establishment of relationships in all areas, or rather a new interaction network.

The networks concept was widely used during the sixties and seventies; from the results of various investigations, other concepts were minted, such as exchange networks and power networks (20, 21). Currently, different applications and meanings are attributed to the network concept, such as information and collaboration networks (14, 15), collaborative social network in the commercial field (11) or production networks (37, 38), which include the Silicon Valley which allow to shorten

the production cycles and technological change, to partnerships with suppliers, collaborative networks, and institutionalized forms of learning. Others are the innovation networks DeBresson and Amesse (1991), Aguilar-Ávila (2010), Aguilar *et al.* (2016, 2017), actors and socio-technical networks (7), knowledge networks (21), marketing networks, financial networks, techniques, and management (27), value networks or cluster (28).

In 2010 Muñoz and Santoyo designed a methodology for networks analysis that has been widely used in Mexico, based on this experience they propose a political and public agenda in relation to the Mexican countryside (30). At the same time, this theory has served as an analytical framework for multiple thematic applications.

"Networks are forms of social interaction, defined as a dynamic exchange between people, groups, and institutions in contexts of complexity. An open and permanent construction system that involves groups that identify with the same needs and problems and that are organized to enhance their resources. A fragmented society in isolated minorities, discriminated against, that has devitalized their networks with citizens lacking protagonism in transforming processes, it sentences itself to a limited democracy. The network intervention is a reflective and organizing attempt of those interactions and exchanges, where the subject is based on himself, differentiating himself from others" (36).

In this article, it is made a study of the possible obstacles that hinder the effective application of rural development policies in a territory, through the networks analysis.

Flores (2007) indicates that Pecqueur (2001) differentiates between a given territory, which is the one that defines a political-administrative decision, and a territory that arises from a meeting of social actors, that is, a determined geographical space that seeks to solve a common problem. This description of territory is similar to the networks definition that is explained as the interaction of actors that meet around a common objective or to solve a common problem. In this sense, the built territory is considered as a space of social relations where there is a sense of belonging, an identity built and associated with the space of collective action, where bonds of solidarity emerge.

For their part, the analysis of graphs allows to identify the different structures of the analyzed networks, to, through the calculation of the basic indicators, identify which are the key actors of

the networks, how the different actors intervene, and what are their organizational arrangements. Specifically, the present investigation is focused on the case of the Yaqui Valley territory, where it is interesting to establish if a deficient structure of networks has been able to cause imbalances in the distribution of aid for rural development.

The analyzed territory corresponds to the Yaqui Valley (figure 1, page 314), partially covers the municipalities of Cajeme, Bécum, San Ignacio Río Muerto, Benito Juárez, Etchojoa, and Navojoa. This territory forms a plain that begins in the foothills of the Sierra Madre Occidental mountain range and extends westward to the beaches of the Gulf of California.

The main activity of the area is agriculture; of the different municipalities that make up the Yaqui Valley, Cajeme stands out for its cultivable area of approximately 81 thousand 750 hectares, of the 220 thousand that this territory comprises. It is located in the center of the region and its head city is Ciudad Obregón.

The studied territory is characterized by a high concentration of public support to the agrarian sector, since 86% of the resources coming from the programs "Oportunidades, Procampo, Agro, Progan", which structure the public policy related to productive supports for the primary sector and have a higher incidence in rural environment in Sonora, that is applied in two regions: Coast Region and Yaqui and Mayo Valley Regions.

In addition, it is estimated that 97.9% of Procampo's support is applied in favor of 10% of the producers with the highest income in the territory, as is the case with 98.6% of other programs such as Agro and Progan (10). This situation exacerbates inequality and poverty conditions in rural areas.

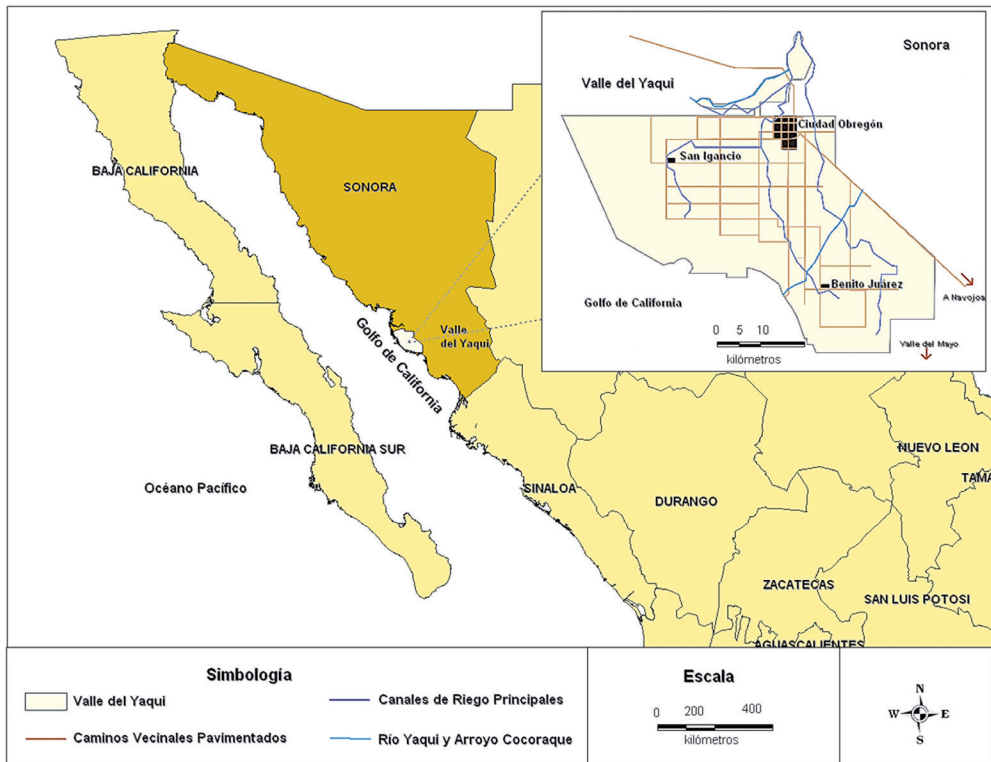


Figure 1. Yaqui Valley territory, Sonora.

Figura 1. Territorio del Valle del Yaqui, Sonora.

The most affected territory includes the Yaqui and Mayo Valleys with 58.7% of households in total poverty and 25% in food poverty (10).

However, this region occupies the second place in the perception of subsidies in the State, as the concentration of aid in higher income producers has already been considered, it prevents balanced development and slows down the progress of the most vulnerable producers.

This situation of asymmetry justifies the need to return to sight at the origin for the analysis of the causes of a situation that is a proof of the lack of effectiveness of a policy that is proposed as an objective

of poverty reduction. It has to do with productivity, but also with redistribution and capacity creation. In this difficult context, the articulation in producers networks of the different sectors (social and private) could favor their situation, allowing them to face in better conditions their access to financing, technical assistance, market, and public support to consolidate itself as a true social network that allows the construction of its territory based on the activity it performs, counting with the structure that favors the conditions necessary for development.

For some authors, network analysis began with the publication of Moreno

(1934) entitled "Who Shall Survive?", who developed a technique for a classification process that is calculated, among other things, to bring together individuals who are capable to have harmonious interpersonal relationships, and thus create a social group that can function with maximum efficiency, and with the minimum of trends and descriptive processes.

The network as a social field where each person is related to a certain number of individuals. A visual representation of these networks is added in which people are represented with points, and relationships between people are represented with lines to each other (24, 25).

The networks can be studied from a national, regional (that is associated to the cluster) or international perspective, which refers to the global chains of products (Carrillo and Novick, 2005). Due to the above, the approaches of Porter (1990) were also used, where networks are formed from the relationship between the actors that are located in a specific territory.

The theory of networks has been applied in various studies on agricultural products in Mexico. These studies combine several analysis techniques, including graphs, which are graphical representations of the network, which are constructed from the nodes (which are individual or collective actors that are represented by geometric figures) and the type of links (consisting of relationships that are established between agents around a common goal), which makes it possible to map the social structure that makes up the territory.

In this difficult context, the articulation in networks of the producers of the called social sector of wheat production in the Yaqui Valley, could favor their situation allowing them to face in better conditions their access both to the market and to public support.

Bott (1990) extended the concept to the economic and labor field, and this reading was considered one of the most advanced contributions of her time in the study of social networks, according to placing them in a social-historical context. The author incorporated the concepts of connectivity (number of connections or amplitude of the network), dispersed networks (with little connection), and high association network (which refers to a greater number of relationships).

Later Vásquez (1999), when applying the concept to territorial development defines the network as "a system of relationships and/or contacts that link the companies or actors to each other whose content can refer to material goods, information, and technology". These relationships can be reciprocal, interdependent, multi-linked, and weak links, and strengthen the network by the exchange of information, as well as by the dissemination of innovation.

For Hannesman (2000), a social network is a set of actors (or points, nodes or agents) between which there are links or relationships. A node is an actor or group of actors represented in a network.

The nodes are usually represented through geometric figures such as circles, triangles or squares. Relationships are represented by lines that connect the nodes. These relationships can be separated into symmetric or directed. A symmetric relationship involves that two actors mention each other; a directed relationship involves that one actor refers to another, but they do not refer to each other. The number of relationships maintained by an actor will define the level of centrality of the same and therefore its influence on the network.

Méndez (2001) indicates from the context of the economic, technological and political-institutional transformations

of the last decades, that innovation acquires a strategic importance and in its promotion the characteristics of each territory, the presence of local actors, and the creation of formal and informal cooperation networks result essential, capable of promoting sustainable territorial development processes.

The author analyzes the theory of the innovative environment and, from it, presents the possibilities and obstacles to promote innovation in peripheral territories dominated by the presence of small companies and traditional activities, suggesting some reflections based on empirical researches conducted in Spain.

According to Casas (2003), networks can be classified by type of morphology, based on two elements: a) the type of involved actors (which can be networks between offerors or providers and users), and b) the objective they pursue, which is reflected in agreements and cooperation agreements, research, and services. The issue is to identify the actors that take initiatives in the construction of networks and the processes that are developed in the conformation, organization, and mechanisms that integrate the networks.

The formation of networks or value chains are conceptually simple but operationally difficult. The difficulty lies in determining where the network starts and ends. The decision will be taken by the researcher and will depend on the research objectives.

Based on the above, it is clear that a loss of social structure and territory deterioration in the Yaqui Valley, in terms of organization in the agrarian and rural areas, hinders the effectiveness and efficiency of agricultural policies with incidence in this territory, originating big imbalances. Added to the inefficiency in the production processes, management,

and irrigation water driving Olmedo Vázquez *et al.* (2017), pose challenges for the Yaqui Valley territory.

In addition to the internal factors that condition the territory, externally it must be adapted like any other member of the WTO and FTA. González *et al.* (2016) points out that in Spain as a product of the WTO negotiations, it has focused its efforts on the search of its internal and external policies based on the sustainable management of natural resources, territorial balance, and rural diversity, taking care of the aspect of food security in a context of market instability.

The current research has been carried out with the general objective of identifying the actors that make up the networks in the Yaqui territory and their change in densities as one of the factors that determine the effectiveness of the policy applied in the territory, comparing two samples of 2006 and 2016. "Linked to this objective, it is proposed to describe the obstacles for the densification of networks that favor the agricultural producers labor in the Yaqui Valley territory".

It is based on the hypothesis that exists a growing disarticulation of the actors that make up the territory networks that favors inequality.

MATERIAL AND METHODS

For the development in the current study, it was carried out in a survey applied to 156 organized and independent producers (73 organized producers and 83 independent producers). This survey was divided into six points that consisted of: 1) Identification, 2) Organizational development, 3) Support, 4) Problems, 5) Innovation dynamics and 6) Network analysis.

The survey was complemented by interviews with organizations representatives, which allowed to understand how the strategies developed in the different networks are working, the integration, and types of networks, number of partners, production surface, technical assistance, obtained volume, product price, local, regional, national, and export market, production costs per cycle; networks advantages, what they do to make them work, infrastructure, and received supports.

To identify the main producer organizations and actors involved in the different networks, visits, interviews, and surveys were carried out with representatives and producers of the following Credit Unions: Yaqui Valley's Agricultors Association, Cajeme's Agricultural Credit Union, S.A. of C.V., Yaqui's Agricultural Credit Union, Yaqui Valley's Agricultural and Industrial Credit Union, Huatabampo's Agricultural Credit Union, Of the Yaqui Mayo Valleys and Guaymas A.R.I.C. of R.I. and Pases A.R., Agricultor Organisms Association of Southern Sonora, Agriculture, Livestock, Rural Development, Fishing, and Feeding Secretariat; Yaqui River's Irrigation District, Grains of Sonora, Tepeyac and Instituted Trusts Related to Agriculture.

When interviewing the producers, they were asked questions that allowed identifying their main suppliers and their customers, which could detect a larger group of actors made up of commercial houses, government institutions, private institutions, credit institutions, industrial, and intermediaries, as well as their social relationship in different events where they attend.

The network mapping and the calculation of its indicators as centrality and density were made through the program "Ucinet 6.8e" Borgatti *et al.* (2002) for network analysis. This program contains a platform for the management of arrays

that represent the interactions between the different actors of a network, as well as allowing to graph the interactions between the actors, which is more accessible and facilitates their interpretation. Each network comes from a matrix composed of rows and columns where the keys representing the surveyed producers and the actors with whom they are related in the different networks are placed.

Depending on the type of relationship, a key was assigned (1: public policy management, 2: financial, 3: technical, 4: management ...), the resulting arrays were initially worked on in Excel and then they were imported into Ucinet where the networks that resulted from the survey applied to wheat producers were elaborated.

The matrix is composed of rows and columns that represent each of the actors identified during the fieldwork phase that includes the 156 producers surveyed; the organization representatives; and to other types of actors such as customers, suppliers, and support institutions that participate in the production process. The columns contain the type of links that the surveyed producers maintain with the actors in the network.

The main basic indicators on which the study focuses for the network analysis are density and centrality. These indicators are applied to the complete network, and for the nodes the centrality, intermediation, and proximity are calculated.

The *density* represents the network connection level, which results from dividing the number of existing relationships between the total of possible relationships in %. This calculation indicates the level of network connectivity.

The *centrality* represents the number of actors, to which an actor is directly linked. The level of centrality is divided into input level and output level. These levels depend on the flow direction.

The *output* level, indicates the number of relationships that the actors say they have with the others. For example, a producer says that he markets through a marketing company, which delivers the wheat production to an agent who is responsible for marketing (producer organization, marketing company, marketing committee, and credit union) or directly to the agroindustrial, for which the output level will depend on the number of agents with whom it is related during the production and commercialization process. The input level is the number of relationships referred to an actor by a surveyed producer.

The network *size* is given by the number of nodes or actors that consist of people or groups of people, which are related to a common goal (public policy management). In the current study, the nodes represent producers and organizations represented with circles.

The *centralization* level indicates how close the network is to behave as a star network, which is negative since it indicates that a single actor controls the entire network. The ideal is to have networks with a low centralization degree well connected where the actors are properly interrelated.

RESULTS

The results obtained in the study are presented in a diagram with the actors and interactions of the Yaqui territory. These actors identified from the interview with key actors and the producers survey from three sectors (private, social, and independent) are those who make up the different types of networks, so that the same actor can appear in several structures performing different functions.

In the table 1, can be seen the different analyzed networks, as well as the nodes number, existing relationships, their possible relationships, and their density, which allows to determine that the five networks have a low density.

Public policy management network

Figure 2 (page 319), shows the main network (general), which allows to determine the disconnection in the truss of studied networks: public, financial, commercial, technical, and social policy management, which are presented with greater force in the private sector.

The networks are exemplified by the role of actors in the Yaqui territory, and most of them were referred by the surveyed actors as sources for agricultural production.

Table 1. Yaqui Valley: nodes number, existing relationships, possible relationships, and their density.

Tabla 1. Valle del Yaqui: número de nodos, relaciones existentes, relaciones posibles y su densidad.

Networks	Nodes	Existing relationships	Possible relationships	Density	Density (%)
Public policy network	492	149	241,572	0.00061	0.061
Financial network	492	137	241,572	0.00056	0.056
Marketing network	492	157	241,572	0.00064	0.064
Technical network	492	174	241,572	0.00072	0.072
Social network	492	79	241,572	0.00032	0.032

Source: own elaboration with field work data, 2016. / Fuente: elaboración propia con datos de trabajo campo, 2016.

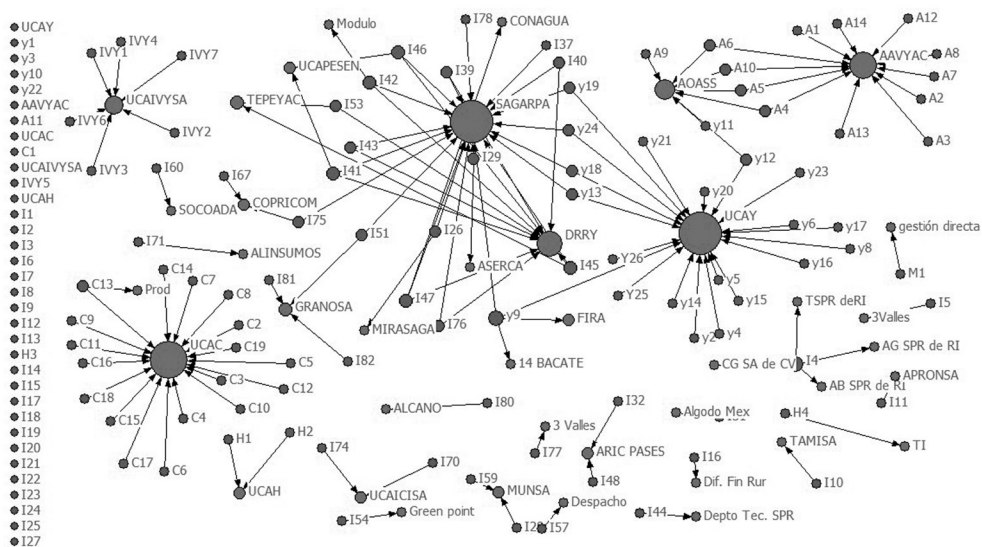


Figure 2. Public policy management network in the Yaqui Valley: Producers network and main actors.

Figura 2. Red de gestión de política pública en el Valle del Yaqui: redes de productores y principales actores.

The fact that the centrality falls on the agricultural policy management network, and is the one that unites the other existing networks, allows to establish that both private producers organizations, as well as the parafinancial companies that operate in the territory, agglutinate the majority of the actors that have a truss with the production link in the agricultural sector; offering different forms of cohesion, with an instability trend.

One of the most important problems is that small producers have less relationship in this network due to various factors such as economic production capacity, a smaller area, lower commitments in leverage, and lower management capacity in all areas, or little interest in having relationships that allow them to streamline their activity.

The public policy management network is characterized by having 492 nodes and 149 relations with a density of social structure in the territory study of 0.061%, obtained from the division of the relationships number over the total of possible relationships, representing a limitation for the management of public policies. This limitation does not allow to articulate the social structure, there are important gaps in the timely support arrival, as well as little or no other supports negotiation that could allow them to have better production conditions, and make their agricultural activity more competitive and profitable.

In terms of its centrality, a way to summarize the relationship patterns to manage programs that structure the support policy such as PROCAMPO and other programs, is by mapping the analysis of the agricultural policy management network, allowing to identify the main actors visually by the node size, which depends on the number of times they were referred, by the interviewed actors, as responsible for managing the supports.

Most of the responsible organizations for managing the various support programs correspond to private sector organizations, listed below are the actors and some institutions, among the most referred are UCAY with 21 references, UCAC 18, AOOAS 7, SAGARPA 21, AAVYAC 12, DRRY 11, UCAYVISA 6, Tepeyac 3, Granosa 3, UCAH 2, FIRA 2 and the rest of the organizations with a single reference which confirms even more the disarticulation with the existing organizations, making the actors their management in a personal way.

It can be determined that the main connections are at the level of captive actors, linked to each of the organizations, meaning a centrality control, which does not allow them to cohere with any other type of truss in the territory. Or, there is a closer influence with their organization than with the others that are in the same territory. Thus, with such disjointed networks it is difficult to accomplish with the territory construction, which is essential for the agricultural management policy for the benefit of the same actors in the territory in general.

Financial network

A main factor that allows the agricultural sector to be active in the territory of study, is the financial network. Therefore its importance, where it is observed that

there are various banking institutions and organizations created precisely to solve the actors need, and there is a link between them; the truth is that once again there is a control centrality, which makes it impossible for the social structure to cohere as can be seen in figure 3 (page 321). This network is characterized by 492 nodes and 137 relationships with a density of social structure in the territory of 0.056%, which reflects the poor articulation of its truss. This network has a great importance, because it is the one that provides the economic resources for all activities, since the production process is integrated by tasks such as soil preparation, planting, fertilization, cultural work, irrigation, and drainage (cost of water, channels cleaning, construction and repair of showers, pre sowing irrigation, aid irrigation, furrows stick).

Since water management, for agricultural production in irrigation district No. 041, Río Yaqui, in northwestern Mexico, is one of the main factors to analyze, to make you efficient and competitive in the activity (Olmedo Vázquez, *et al.* 2017), plagues and diseases control, harvest, and commercialization. All these processes are not only complex, but have a high production cost derived from the inputs and labor required to carry them out.

Commercial network

In relation to the results obtained for this network, the existence of a poorly articulated network was detected, which does not necessarily represent a weakness, since it allows for greater interaction between different actors, and producers have the possibility of establishing marketing agreements and arrangements with the actors that provide the greatest support for production and commercialization.

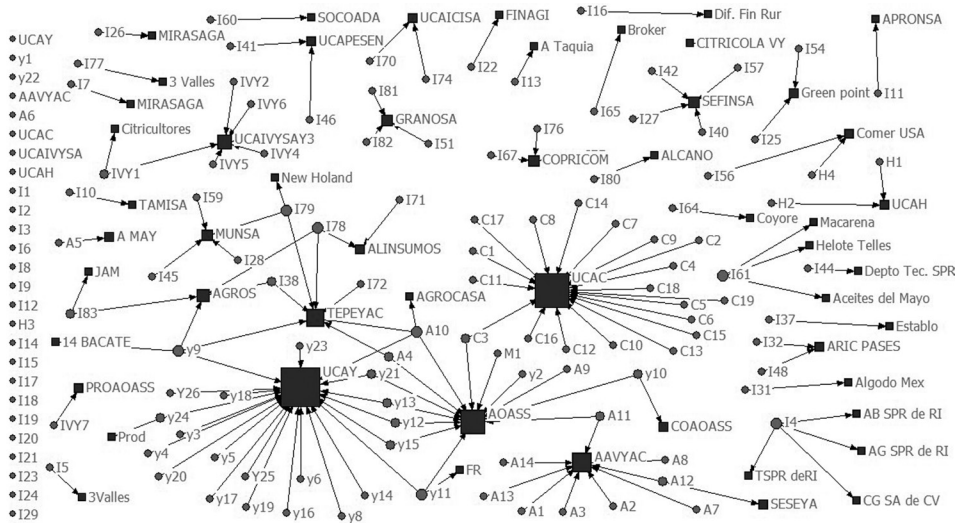


Figure 4. Marketing network in the Yaqui Valley: producers network and organizations.

Figura 4. Red de comercialización en el Valle del Yaqui: redes de productores y organizaciones.

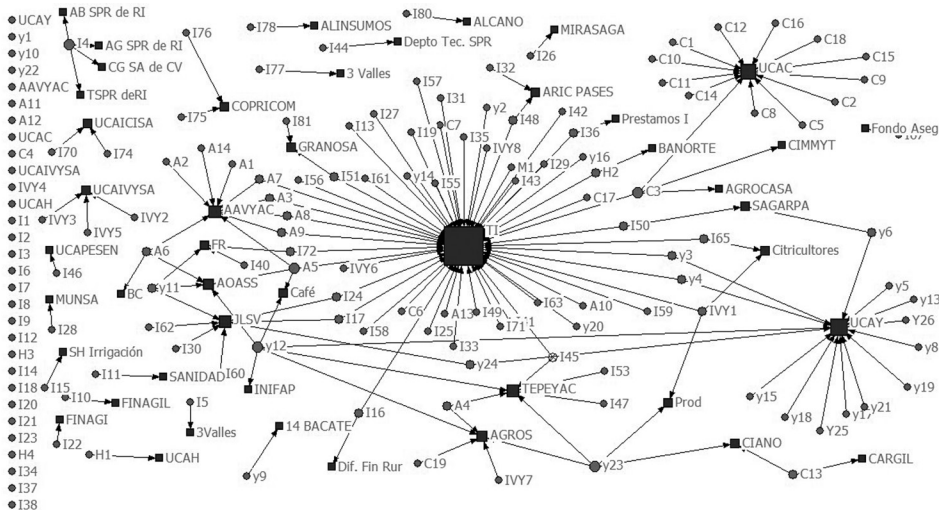


Figure 5. Technical Network in the Yaqui Valley: producers network and organization.

Figura 5. Red Técnica en el Valle del Yaqui: redes de productores y organizaciones.

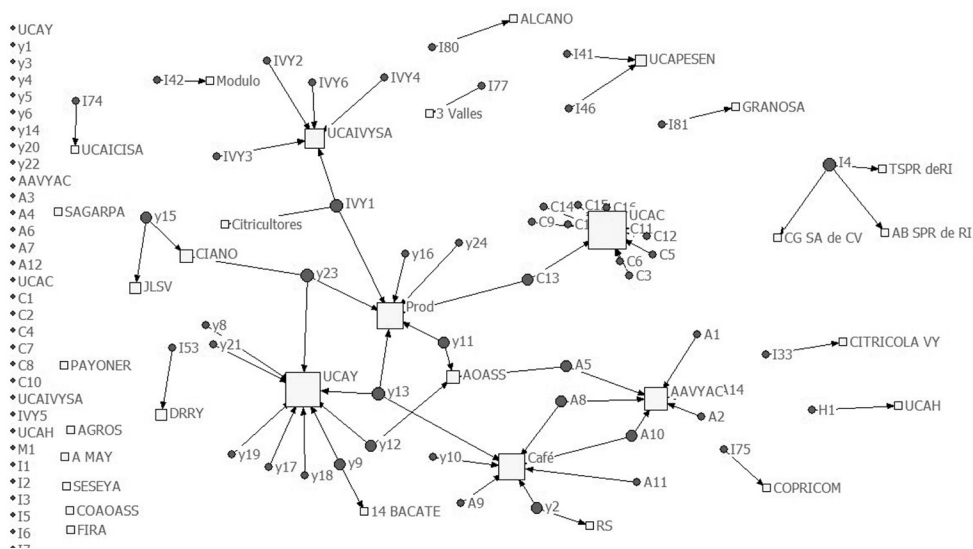


Figura 6. Social Network in the Yaqui Valley: producers network and organization.
Figura 6. Red Social en el Valle del Yaqui: redes de productores y organizaciones.

When disconnecting these nodes most of the existing relationships are lost. An important node in this network is coffee as a meeting point where experiences are shared, related to the activities carried out by the actors.

Although the social network analysis method uses a vocabulary to describe connectivity and distance, it is, in fact, very simple and easy to learn the properties of a network, for example the density of 0.032% represents a low density, and the level of connection of the network of actors. These are the relationships between the actors that make up the network that can be driven, having multiple links, the information that can be extracted from this network is that no reciprocal relationships are observed, where most of the actors are disconnected.

Boza *et al.* (2016), considers that it is necessary to identify and analyze the

producers attitudes in the face of the public policy actions carried out or to be carried out, it constitutes a relevant tool in its design, monitoring, and evaluation; that strengthens the social network of the territory. Sánchez-Toledano *et al.* (2017) indicates that, if one intends to understand the adoption behavior of technological innovations, it is necessary to understand the farmers heterogeneity considering not only their socioeconomic characteristics and those of their farms, but also their opinions, attitudes, preferences, and objectives.

Successive times

Machín (2011) recommends compiling information for successive times, with the purpose of having a representation in the temporal evolution. In this study, field data are available for the year 2006 and 2016; from which the networks were mapped, and their indicators were

calculated in UCINET-6 in order to make a comparison of the densities of the social structure. This is due to the fact that the territory was defined as a social construction, where the networks theory and the graph method allow to analyze the characteristics of said structure. The graphs represent an intuitive observation and in constant movement.

The density shows us the high or low connectivity of the network expressed as a percentage of the quotient between the number of existing relationships with the possible ones. In the case in question, the density is 0.34 in 2006 and 0.06 in 2016 with 149 leagues and a standard deviation of 0.025.

The network cohesion level is reduced by the decrease in the total average density of 24%. That is distributed in a fall of 52.47% in the technical network density, 75% in the financial network, 86.41% in the commercial network, and 81.79% in the agricultural policy management network, unlike the social network that registered 76.21% of increasing, however, it is the smallest network and less leagues, in 2006 it was scarcely linked.

This sequence clearly shows the disarticulation of the networks; especially in the case of the social sector where

most of the organizations identified in 2006 do not appear in 2016. In addition, networks of lower density were found than those detected in the study of 2006, which represent the restructuring of the social structure in the territory; where the causes can be multiple, for example: deficient and delayed supports, concentrated in a small sector that has the highest territory incomes. The effects can be also multiple, so it is necessary to analyze them to counteract the territory deterioration.

In the table 2, shows a percentage value comparison, and represents the deterioration of the social structure of the territory over a period of ten years.

The reduction of density in most of the networks that make up the territory is an indicator of low connectivity, whose causes and socioeconomic effects and in terms of agricultural policy management must be analyzed.

Based on the results of the survey applied to 156 actors, who are part of the Yaqui Valley networks, they point out among the main obstacles for the network densification, the high production costs (85.9%), low prices of crops (83.97%), late and insufficient support (81.41%), obsolete machinery (64.10%), low labor availability (58.97%), credit availability (58.3%), among others.

Table 2. Density by type of network (technical, financial, commercial, policy, and social) 2006, 2016.

Tabla 2. Densidad por tipo de red (técnica, financiera, comercial, política y social) 2006, 2016.

Year	Technical	Financial	Commercial	Policy	Social	Average
2006	0.15	0.23	0.48	0.34	0.01	0.24
2016	0.07	0.06	0.06	0.06	0.03	0.06
Rate of exchange (%)	-52.47	-75.56	-86.41	-81.79	176.21	-24.00

Source: elaborated with field data in the Yaqui Valley, 2006 y 2016.

Fuente: elaborada con datos de campo en el Valle del Yaqui, 2006 y 2016.

Centrality

To identify through whom the agricultural policy supports are managed, and based on the applied survey in 2006 and 2016, the central actors were identified by the greatest number of relationships. These actors were the most referred by the survey respondents as those through which the agricultural policy supports are managed.

In this sense, it was found that most of the survey respondents manage their support through the organization to which they belong and in some cases they manage directly with the responsible institution such as SAGARPA, ASERCA, however, the majority refers to the organization among those that stand out the organizations of the private sector (table 3).

Table 3. Yaqui Valley: agricultural policy management network and central actors 2006 y 2016.

Tabla 3. Valle del Yaqui: red de gestión de política agrícola y actores centrales 2006 y 2016.

Actor	2006	Actor	2016
ASERCA	52	UCAY	21
SAGARPA	45	SAGARPA	21
GE	7	UCAC	18
UCAC	6	AAVYAC	12
UCAY	5	DRRY	11
GF	5	AOASS	7
PASES	4	UCAIVYSA	6
UCAIVYSA	4	TEPEYAC	3
AAVYAC	3	GRANOSA	3
Social Company	3	UCAH	2
FIRA	3	FIRA	2
G 3 VALLES	2	MUNSA	2
COPRICOM	2	ARIC PASES	2
FP	1	UCAPESEN	2

Source: elaborated with field work, 2006 y 2016. /
Fuente: elaborada con trabajo de campo, 2006 y 2016.

Centralization

The 100% centralization index indicates that there are many more central actors than others, and it resembles the behavior of a star network. On the other hand, completely the opposite, to the star network would be low values in this indicator, which would indicate the absence of clearly central actors.

Within the analysis of networks there are input and output centralization levels; the use of one or the other will depend on the need for specific results of each network.

The table 4 presents the results of the input and output centrality levels of the agricultural policy management network and it is observed that in 2006, the network was closer to behaving as a star type network with a centralization level of 50% compared to 4.22% registered in 2016.

The first implies that one or two actors play a central role in the control of the entire network; unlike the 2016 that is far from that behavior, which is more favorable because this allude of a better connected network.

Table 4. Yaqui Valley: centralization of the policy management network, 2006 y 2016.

Tabla 4. Valle del Yaqui: centralización de la red de gestión de política, 2006 y 2016.

Centralization	2006	2016
	[%]	
Output	3,576	2,795
Input	50,106	4,224

Source: elaborated with field work 2006 y 2016.
Fuente: elaborado con trabajo de campo 2006 y 2016.

DISCUSSION

To interpret the territory it is necessary to understand the way it emerges, the networks that sustain it, the organizations, groups or networks of intertwined firms within the production systems; and that depend not only on the territory context but also on the greater or lesser relationship that the actors maintain among themselves.

Territories can be characterized by strong or weak and scattered relationships. Where the subjects of the process should be seen as reflexive human actors both individually and collectively (39).

All the identified networks in the research present a low articulation measured from the densities that according to Wron (1961), represents weak ties, which it is not necessarily negative but can serve as a channel for the emergence of new structures and from the economic point of view a society with many networks can be more efficient than a society with many static networks (16).

Based on the results of the comparative analysis in the 2006 and 2016 densities, and given the average reduction of 24%, the hypothesis that exists a growing disarticulation of the actors that make up the networks that favors inequality in the territory is corroborated.

CONCLUSIONS

The studied territory has very few relationships among the main actors, so with networks of such characteristics it is very difficult to build a vision of short, medium, and long term commitments. It is essential to strengthen the bonds between

the actors that make up the different networks, otherwise the territory will continue to suffer the effects of disarticulation in space and time, where local actors can take effective actions to build a socially fair territory, economically viable and suitable to live in harmony.

It is necessary to strengthen the networks of local actors, enabling them to design support plans and programs based on the territory potential, and the objectives agreed upon by their members; counting on a dense social structure and promoting articulation in the various socioeconomic sectors that are able to reconstruct the structure, and turn it into a territory with greater opportunities for social equality.

Considering the type of structure of the networks that make up the socio-economic structure of the territory under study, such as producer organizations, where the private sector creates instances at the mesolevel through lobbying practices in federal offices directly, which can exclude others within the territory itself, without the need for the intervention of a dominant group or external factors; since the actions at the national level determine the results at the territorial level, and reinforce the stereotypes that in the light of the work that concerns us is represented by the agricultural programs and policies that arrive to the territory, which reproduce and reinforce the conditions of inequality in the rural environment.

By continuing to rely on networks that are disjointed, and that due to their low density are unable to manage better support or affect the agricultural policies of the federal government, the benefit of the territory in general will be untenable.

The networks found in the territory of the Yaqui Valley have been disarticulated

over time, because ten years after being analyzed for the first time, the calculation of densities has been reduced by 24% on average, and the truss of the social sector practically tends to disappear.

To improve the networks articulation, it is necessary to eradicate the main obstacles; as: high production costs, low prices of crops, late and insufficient support, obsolete machinery, low labor availability, credit availability, among others.

It should be noted that the relationship between poverty and social inequality is

complex; since a reduction of the first is compatible with a significant increase of the second (19).

The wealth concentration in the Yaqui Valley, analyzed through the different types of income, government support, and land resources, is compatible with a low density of the networks. Apparently there is a greater concentration of resources in a smaller number of actors in the network, however, this should be tested in a forthcoming study.

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***Puccinia meyeri-albertii* in leaf and fruit of calafate (*Berberis microphylla*) in La Araucanía Region, Chile**

***Puccinia meyeri-albertii* en hojas y frutos de calafate (*Berberis microphylla*) en la región de La Araucanía, Chile**

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ABSTRACT

Calafate (*Berberis microphylla* G. Forst) is an endemic species of the Patagonian Andes of Chile and Argentina, with potential use for agroindustry. One aspect that could be restrictive for the production is the Calafate rust (*Puccinia meyeri-albertii* P. Magn), which the last reference in Chile dated from 1945. The objective of this study was to identify and updated report about to the prevalence of Calafate rust in La Araucanía Region, Chile. The rust identification was consistent as that reported for *P. meyeri-albertii*, aspects also confirmed by Centre for Agricultural Bioscience International (CABI) under number IMI-50016. The sequence amplified of the 28S rDNA (accession number KY555071) had an identity of 97% with others six *Puccinia* species, likewise, the genetic relationship analysis established that *P. meyeri-albertii* had higher divergences in the nucleotide substitutions respect to the others rusts; it consign that no nucleotide sequences available in NCBI database for *P. meyeri-albertii*. Prospecting realized the 2011-2012 seasons in an experimental Calafate orchard, the incidence and severity were respectively: 24.70% (range 4.72-36.90%) and severity 2.97 (2.15-3.30, range affected area) in leaves; and in fruits 13.78 % (range 1.13-23.84%) and 2.40 (2.01-2.78, range affected area). Hence, it was suggested that it is necessary to develop molecular, epidemiological and control research, as also to determine the infection level of this pathogen in others Calafate ecotypes and *Berberis* species growing in different edaphoclimatic conditions.

Keywords

Berberis • Calafate • *Puccinia meyeri-albertii* • rust

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RESUMEN

El Calafate (*Berberis microphylla* G. Forst.), es una especie endémica de Los Andes Patagónicos de Chile y Argentina, con potencial de cultivo para la agroindustria. Un aspecto que pudiere ser restrictivo para su establecimiento y producción es la roya del Calafate, cuya última referencia en Chile data del 1945. El objetivo de este estudio fue identificar y actualizar la prevalencia de esta roya en la Región de La Araucanía, Chile. La identificación morfológica fue consistente con *Puccinia meyeri-albertii* P. Magn, determinación corroborada por el *Centre for Agricultural Bioscience International* (CABI) y registrada con el número IMI-50016. La secuencia amplificada desde el 28S del ADN ribosomal (accession KY555071), tuvo una identidad de 97% con otras seis especies de *Puccinia*. El análisis de relaciones genéticas de *P. meyeri-albertii*, representadas en un árbol filogenético, generó amplias divergencias sobre las sustituciones de nucleótidos respecto de las otras royas comparadas en este estudio; es de consignar que en la base de datos del NCBI no se dispone secuencias nucleotídicas para este hongo. A través de prospecciones realizadas durante la temporada 2011-2012 en un huerto experimental de calafate, la incidencia y severidad de *P. meyeri-albertii* fueron respectivamente: 24,70% (rango 4,72%-36,90%) y 2,97 (2,15 - 3,30, rango de área afectada) en hojas; mientras que en frutos fue de 13,78 % (1,13-23,84%) y 2,40 (2,01-2,78, rango de área afectada). Al respecto, sería conveniente generar información en aspectos moleculares, epidemiológicos y de control de este patógeno, como también evaluar el nivel de infección en ecotipos de calafate y especies de *Berberis* que crecen en diversas condiciones edafoclimáticas.

Palabras clave

Berberis • Calafate • *Puccinia meyeri-albertii* • roya

INTRODUCTION

Calafate (*Berberis microphylla* G. Forst.) belongs to the Berberidaceae family, which contains around of 15 genus and 650 species distributed worldwide. In Chile, this genera it is represented by 20 species (10, 19). It is a perennial spiny shrub, growing to a height of two metres or more (16). Calafate grows in the different altitudes on pre-cordillera, in moist steppe areas, along the river and stream banks from Del Maule to Magallanes and Chilean Antarctica regions (6, 9).

The scientific information in Chile about *Puccinia meyeri-albertii* associated to Calafate is scarce. The first reference is comprise

in "*Flora Fungosa Chilena*" (13) associated to the Calafate (*B. microphylla*) and also other species belonging to Berberidaceae family (i.e. *B. blaurina* Bellb., *B. chilensis* Gill, *B. darwinii* Hook, *B. linearifolia* Ph. and *B. congestiflora* Gay). Other historic and relevant reference is that mentioned in "The Repertoire of Literature Cryptogamic" (1892) associated with *Berberis* genus growing in South America (14).

The pathogenic and epidemiological information regarding *P. meyeri-albertii* is scarcely and principally associated with *Berberis* genus (5, 13, 21).

Recently, there has been increasing interest for commercial use of its fruits, which can be consumed fresh or in processed foods (jams, jellies, and beer) (4, 20) due to its high level of antioxidants as well as alkaloids of the berberine group (3, 12, 18).

The quality and condition of Calafate fruit for the industrial process could decrease due to the severity of the attack of *P. meyeri-albertii*. In this sense, as firstly required identify causal agent of the Calafate rust, and quantify the prevalence in ecotypes, seasons and different environment conditions, to design control strategies for this pathogen in endemic plant species from Chile and Argentina, which last reference in Chile dated from 1945. Hence, the objective of this study was to identify and updated report about the prevalence of Calafate rust in La Araucanía Region, Chile.

MATERIAL AND METHODS

Sample collection and geographical location

Symptomatic leaves and fruits were collected every 15 days in 2011-2012 growing seasons from Calafate plants of 10 years localized in the Maquehue Experimental Station (38°50' S, 72°41' W), belonging to Universidad de La Frontera.

The symptoms in leaves corresponding to a yellow discolouration, reddish brown patches, and dehydration of the fruits and signs are orange uredosoric pustules and brown teleutosoric pustules. The samples collected from different twigs (n=10) homogeneously distributed in the plant were maintained under refrigeration at 4°C for approximately seven days until their analysis in the plant pathology laboratory of Instituto de Agroindustria, Universidad de La Frontera.

Morphometric and molecular features

The teliospores (n = 30) were measured according to described by Zuluaga *et al.* (22, 23).

The shape, colour, dimensions, apical and lateral wall size, ornamentation, pedicel length and the viability of the teliospores, and also basidiospores were morphometrically characterized using a Carl Zeiss stereoscopic microscope (model Stemi DRC) and a Nikon optical microscope (model Alphaphot-2 YS2-Hse).

For the germination of teliospores, a suspension using 10 ml with sterile distilled water was homogenised in test tubes for five-minutes, and placed on Petri dishes with water agar culture medium (2%). The dishes were incubated (20 ± 2°C; RH 90%) and evaluated at 24-hour intervals during three days (data not shown). To confirm the morphological identification, leaves and fruit samples infected were sent to the Centre for Agricultural Bioscience International (CABI), United Kingdom.

Aeciospore masses removed from sorus growth on Calafate fruits were kept into a mortar followed by frozen using liquid nitrogen and crushed with a pestle.

The powder was transferred to a 1.5 mL Eppendorf tube continuing with the manufacturer's protocol for DNA extraction with the Qiagen Plant Mini kit (ref. 69104). PCR reactions were processed in My Cycler™ thermal cycler (BioRad) including 25 µL reaction volume, where: 1x GoTaq Flexi Buffer (Promega, cod. M890A), 2 mM MgCl₂, 0.4 mM dNTPs, 1 µM for each primer, 1 U GoTaq® Flexi G2 DNA polymerase (Promega, cod. M780B) and 2 µL DNA template. Large-subunit ribosomal RNA gene (LSU rDNA) was amplified by both NL1 (5' -GCATATCAATAAGCGGAGGAAAAG- 3')

and NL4 (5'-GGTCCGTGTTTCAAGACGG-3') primers. The thermal profile consisted in initial denaturation step (94°C, 2 min) followed by 31 cycles at 94°C for 1 min, 57°C for 30 s, 72°C for 30 s, and a final extension step for 7 min at 72°C.

The PCR product was observed by 1% agarose electrophoresis gel stained with ethidium bromide in 1x TAE buffer and visualized under UV light. The amplicon was purified using QIAquick® PCR Purification Kit (Qiagen, cat. 28104), and sequenced at Unidad de Secuenciamiento Automático at Pontificia Universidad Católica de Chile. Nucleotide sequence was edited using Lasergene software (DNASTAR, Inc. Madison, WI U.S.) and compared with homologous sequences of *Puccinia* species by Basic Local Alignment tool (BLAST) (1) in National Center for Biotechnology Information website (NCBI, <https://www.ncbi.nlm.nih.gov/>).

A final multiple alignment performed by Jotun Hein algorithm (1990) in MegAlign program, it compared 28S rDNA sequences for 12 *Puccinia* species; this analysis considered segments of 510-nucleotides accurately chosen for matched. Likewise, based in the alignment precedent were established the genetic relationships among the diverse taxons building a phylogenetic tree with parameters of pairwise sequence divergence. *Phragmidium violaceum* (JN790619) was added as an outgroup specie.

Incidence and severity

The incidence of Calafate rust was evaluated in three plants quantifying 100 leaves and 100 fruits for each plant. The frequency of uredosoric pustules were expressed as a percentage using the following formula: Incidence (%) = affected organs/total organs (healthy + diseased) x 100.

To determine the magnitude or severity, the area affected in the same leaf and fruit was estimated by a scoring system to indicate the degree of infection, where: 1 (apparently healthy), 2 (1-25%), 3 (26-50%), 4 (51-75%) and 5 (76-100%). Incidence and severity were expressed as monthly average (figure 1, page 335).

RESULTS

Morphometric and molecular features

The teliospores were bicellular, cylindrical or ellipsoidal, ochre colour, pointed at the distal end, semi-contracted in the area of the division, with slightly pigmented hyaline walls and germination pores in the apical and basal cells semi-contracted in the area of the division from the basal or septal cell, broadening in the centre (figure 2, page 335). The characteristics recorded for the teliospores, with a length greater than 80 µm, thickened wall in the apex, and pedicel over 100 µm (table 1, page 336).

Occasionally, were observed basidia (6-10 µm diameter; average length 8.12 µm (n = 20)) with spherical-ovoid and hyaline basidiospores (figure 2, page 335).

The morphometric analysis of the uredospores is included in table 2 (page 336) and figure 3 (page 335).

The uredosoric phase in Calafate leaves presented exponential growth, declining at the end of summer when they turned brown and hard. Uredospores presented different degrees of dehydration with thick and uneven walls. These morphometric characteristics corresponded to that mentioned by Hennen *et al.* (2005) and Lindquist (1978) for *Puccinia meyeri-albertii* P. Magn.

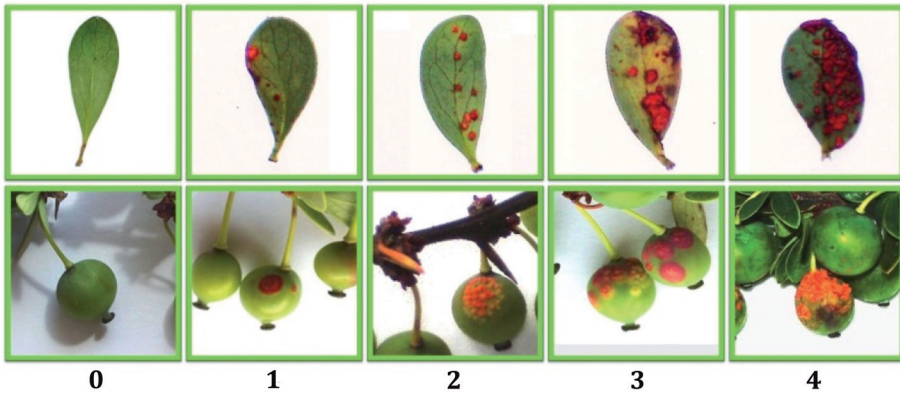


Figure 1. Average severity of *P. meyeri-albertii* in leaf and fruits of Calafate based on the note scale from 0 to 4.

Figura 1. Escala de notas (0 a 4) para estimar severidad de *P. meyeri-albertii* en hoja y fruto de Calafate.



Figure 2. Teliospores of *P. meyeri-albertii*: Mature and germinated teliospores, basidia and basidiospores in germinated teliospores. 100x. (yellow bar = 10 μ m).

Figura 2. Teliosporas de *P. meyeri-albertii*: teliosporas maduras germinadas, basidios y basidiospora en teliosporas germinadas. 100x. (Barra amarilla = 10 μ m).



Figure 3. Uredospores of *P. meyeri-albertii* (yellow bar = 10 μ m). 100x.

Figura 3. Uredosporas de *P. meyeri-albertii* (Barra amarilla = 10 μ m). 100x.

Table 1. Measurement (μm) of length, width, apex, pedicel and full length of teliospores of *P. meyeri-albertii*.

Tabla 1. Medidas (μm) de largo, ancho, ápice, pedicelo y largo total de teliosporas de *P. meyeri-albertii*.

	Teliospores (μm)				
	Length	Width	Apex	Pedicel	Full length
Minimum	58.0	10.0	4.0	70.0	134.0
Maximum	90.0	16.0	14.0	180.0	260.0
Average	74.2	13.6	8.9	127.4	201.6
SD*	7.64	1.8	2.6	26.9	28.2

* Standard deviation; n = 30.

* Desviación estándar; n = 30.

Table 2. Measures (μm) uredospores of *P. meyeri-albertii* P. Magn.

Tabla 2. Medidas (μm) de uredosporas de *P. meyeri-albertii* P. Magn.

Parameter	Uredospores (μm)	
	Diameter	wall
Minimum	20.0	2.0
Maximum	32.0	4.0
Average	25.9	2.5
S.D*	3.44	0.9

* Standard deviation; n = 30

* Desviación estándar; n = 30.

The preliminary identification corroborated by International Centre for Agricultural Bioscience International (CABI-UK) was registered under the number IMI-500169.

The sequence amplified of the 28S rDNA of 655 bp (accession number KY555071) -where, no sequences available in NCBI database- it had an identity of 97% with *Puccinia andropogonis* (GU057993.1), *P. recondita* (KX036373.1), *P. striiformis* (DQ417407.1), *P. triticina* (DQ664194.1),

P. windsoriae (GU057995.1) and *P. graminicola* (KX190872.1). The phylogenetic tree (figure 4, page 337) analysis permitted to establish 13 lineages showing a genetic relationship closest among the *Puccinia* species; at the same time, it was evidenced that *P. meyeri-albertii* had higher divergences (nucleotide substitutions) respect to the others rusts.

The percentage identity of *P. meyeri-albertii* with the others *Puccinia* spp. compared in this study, ranged from 96.9% to 98.6%, wherever the score divergences 1.4 to 3.2 (figure 5, page 338).

Incidence and severity in leaves and fruits

The mean annual incidence in leaves was 25.43 %, with minimum 4.72% and maximum 36.90 % recorded in September and December respectively.

The incidence was higher in younger than mature leaves. At the end of summer, the uredosori in senescent leaves ceased production of uredospores and entered a stage of latency in the form of sub-epidermal mycelia characterized by brown patches (table 3, page 339).

At the end of winter, signs of teleutosore were growing on the abaxial side of senescent Calafate leaves. They were round to irregular in shape, scattered or confluent, exposed, compact, and brown in colour turning greyish with teliospore germination.

The symptoms were yellowness and necrotic patches on the leaf. In leaves, the average notes of severity were between 2.15 and 3.30 (25%-50% leaf area affected); the lowest score recorded in September and the highest in November and December.

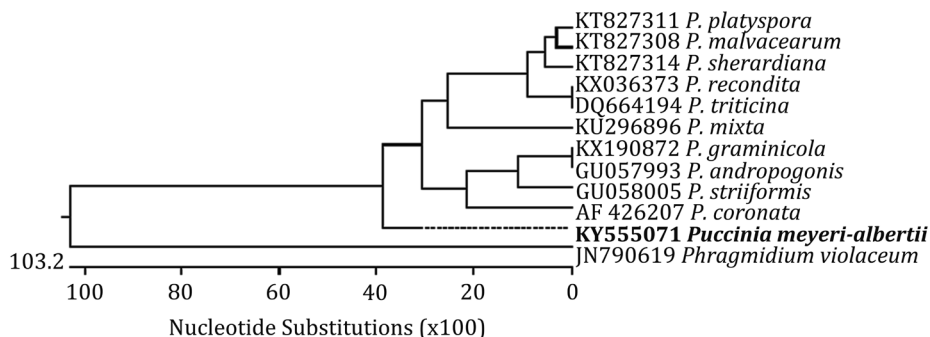


Figure 4. Phylogenetic tree of homologous species to *P. meyeri-albertii* based in the partial sequences of the 28S rDNA (fragment of 510 nucleotides). The relative length of the branches denotes the distance between pair sequences. The units at the bottom of the tree indicate the number of substitution events.

Figura 4. Árbol filogenético de especies homólogas a *P. meyeri-albertii* basado en secuencia parcial 28S del ADN ribosomal (510 nucleótidos). La longitud de los brazos denota la distancia entre pares de secuencias. Las unidades bajo el árbol indican el número de eventos de sustituciones.

Calafate rust infection predominate from September to the beginning of April, being most susceptible in October and November when the pathogen can infect active growing plant tissues (table 3, page 339).

The mean incidence of rust on fruit was 12.43%, from 1.13% recorded in January associated with fruit formation, whereas that highest incidence (23.84%) occurred in mid-spring (November) when the fruit is in the process of ripening (table 3, page 339).

In immature fruits infected was associated yellow-ochre discolouration and concentric ring surrounded for a reddish halo with radial growth.

In the other hand, in advanced stages of infection the symptoms corresponded to fruit deformation, mummification and abscission (berry and peduncle) (figure 6, page 339).

Pustules observed in the fruit from October, increased their number and size as resulted of the reinfection by uredospores.

Nevertheless, the severity did not influence the highest degree on the scale (score 4 = 76 - 100 %, affected area) due to the brief period of fruit formation (October - December). The severity average was the fruit 2.01 in January and 2.65 in December for the same year (table 3, page 339).

DISCUSSION

Species of genus *Puccinia* affecting a broad host range of the genus *Berberis* (2, 21, 24).

On these fungi, the life cycle corresponds to a hemiform rust evidencing only teliospores and uredospores stages (2, 17).

The findings of this study suggest that the uredosporic phase appears from October in younger leaves and immature fruits, after declining towards February giving way to the telial and basial phases in mature leaves.

The symptoms on Calafate leaves were yellowness, reddish adaxial patches, orange abaxial pustules and defoliation. Whereas in fruits were observed orange pustules with different size, dehydration and abscission of fruits (figure 6, page 339). *P. meyeri-albertii* in Calafate had an average incidence of 24.7 % and 13.8 % on fruits in leaves, respectively. Meanwhile average severity scored of 2.9 and 2.4 on leaves and fruits. Spatial analysis studies are fundamental in order to plan strategies to manage crop health and improve sampling methods (15).

Considering the precedent results with implication of local weather conditions, the Calafate plants were most susceptible to *P. meyeri-albertii* infection from October

until December, situation attributed to adaptation and colonization of the rust on younger tissues, situation eventually related with the thickening of the cuticle in mature tissues. We consider appropriated mentioning that *P. meyeri-albertii* was also identified on fruits of Michay plants (*Berberis darwinii* Hook) nearly localized to the study area (data not shown).

The morphometric identification of *Puccinia meyeri-albertii* P. Magn. affecting leaf and fruits of Calafate, it was registered by CABI under number IMI-500169 (2014), determination similar that those previously realized during the year 2010 (IMI-398821), updating the information referenced in Chile from 1945.

		Percent Identity											
		1	2	3	4	5	6	7	8	9	10	11	12
Divergence	1		96.9	98.0	98.6	98.0	98.0	98.2	98.0	97.6	98.4	98.6	90.1
	2	3.2		98.2	98.0	98.2	98.0	97.2	97.4	96.9	98.2	98.0	89.7
	3	2.0	1.8		98.2	100.0	98.6	98.2	98.4	97.6	98.4	98.2	89.7
	4	1.4	2.0	1.8		98.2	98.8	98.8	98.6	98.6	99.0	100.0	90.7
	5	2.0	1.8	0.0	1.8		98.6	98.2	98.4	97.6	98.4	98.2	89.7
	6	2.0	2.0	1.4	1.2	1.4		98.4	98.2	97.8	98.8	98.8	90.3
	7	1.8	2.8	1.8	1.2	1.8	1.6		99.4	99.0	98.2	98.8	90.5
	8	2.0	2.6	1.6	1.4	1.6	1.8	0.6		98.8	98.4	98.6	90.3
	9	2.4	3.2	2.4	1.4	2.4	2.2	1.0	1.2		97.6	98.6	90.9
	10	1.6	1.8	1.6	1.0	1.6	1.2	1.8	1.6	2.4		99.0	90.9
	11	1.4	2.0	1.8	0.0	1.8	1.2	1.2	1.4	1.4	1.0		90.7
	12	10.7	11.1	11.1	10.0	11.1	10.4	10.2	10.4	9.8	9.8	10.0	

1: KY555071 *Puccinia meyeri-albertii* / 2: AF426207 *P. coronata* / 3: KX190872 *P. graminicola*
 4: DQ664194 *P. triticina* / 5: GU057993 *P. andropogonis* / 6: GU058005 *P. striiformis*
 7: KT827308 *P. malvacearum* / 8: KT827311 *P. platyspora* / 9: KT827314 *P. sherardiana*
 10: KU296896 *P. mixta* / 11: KX036373 *P. recondita* / 12: JN790619 *Phragmidium violaceum*

Figure 5. Divergence and identity values of each sequence pair based in the multiple alignments by Jotun Hein method. The divergences have been calculated comparing sequences pairs, wherever the percent identity relating directly the sequences.

Figura 5. Valores de divergencia e identidad de cada par de secuencias basado en alineamientos múltiples empleando el método de Jotun Hein. Las divergencias fueron calculadas comparando pares de secuencias, mientras que el porcentaje de identidad, relacionando directamente las secuencias.

Table 3. Month mean incidence (%) and severity (score) of rust *P. meyeri-albertii* in leaves and fruits of evaluated Calafate.

Tabla 3. Incidencia (%) y severidad (escala de notas) promedio mensual de *P. meyeri-albertii* en hojas y frutos de Calafate.

Month	Incidence (%)		Severity (score)	
	Leaf	Fruit	Leaf	Fruit
November	18.59	12.95	2.78	2.78
December	28.23	6.49	3.08	2.21
January	29.52	1.13	3.06	2.01
February	34.61	-	3.09	-
March	26.34	-	3.00	-
April	19.35	-	2.95	-
May	-	-	-	-
June	-	-	-	-
July	-	-	-	-
August	-	-	-	-
September	4.72	-	2.15	-
October	24.25	7.76	2.94	2.40
November	31.82	23.84	3.30	2.40
December	36.90	22.40	3.30	2.65
Average	24.68	13.78	2.97	2.40

*(-) Asymptomatic tissue or absence of uredosore. n = 32.

*(-) Tejido asintomático o ausencia de uredosoros. n = 32.



Figure 6. Symptoms and signs of *P. meyeri-albertii* on Calafate fruits.

Figura 6. Síntomas y signos de *P. meyeri-albertii* en fruto de Calafate.

The nucleotide sequence determined in the 28S RNA gene showed a plausible preliminary identification complementing the morphometric characteristics. For all these, it is necessary to continue researching about the genetic features due to that there is yet scarce studies about *P. meyeri-albertii*.

The disease should imply a risk factor due to its high prevalence, causing

significant economic losses for Calafate commercial production system.

In addition, it would be convenient to develop epidemiologically and control research, in order to determine the infection level of this pathogen in other species and ecotypes *Berberis* in different edaphoclimatic conditions.

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Eficiencia de pulverizadores hidroneumáticos de uso fitosanitario en la fruticultura cuyana (Argentina)

Efficiency of air assisted sprayer of phytosanitary use in fruitgrowing in Cuyo (Argentina)

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RESUMEN

La protección fitosanitaria de los montes frutales se efectúa principalmente con equipos hidroneumáticos. Debido a la ausencia de estudios actuales, se planteó el objetivo de evaluar el desempeño de los principales equipos utilizados en montes frutales en Cuyo (Argentina). Las mediciones se realizaron a régimen estandarizado de la toma posterior de potencia del tractor. Se evaluó el caudal de aire en túnel de viento y la distribución espacial de la velocidad de aire con anemómetros de hilo caliente. La determinación de la distribución vertical de pulverizados se realizó en banco de lamelas. Complementariamente se determinó la demanda de potencia. Los caudales de aire variaron entre 257,49 m³.min⁻¹ y 485,17 m³.min⁻¹. Los equipos B y C en ambos accionamientos presentaron asimetría en la distribución de aire. La distribución vertical de pulverizados presentó simetría entre el sector izquierdo y derecho, excepto para el equipo B en sus dos accionamientos y E alta. La demanda de potencia alternó entre 11,96 kW y 31,17 kW. Los equipos más eficientes resultaron A en el único accionamiento disponible, D baja y alta y E baja. Los equipos difieren en sus capacidades aerodinámicas, tanto en caudal de aire erogado como en su distribución bilateral y en la demanda de potencia.

Palabras claves

pulverizadora hidroneumática • potencia • caudal de aire • eficiencia

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ABSTRACT

Phytosanitary protection of fruit crops is mainly done with axial fan sprayers. Due to lack of up-dated studies, the objective was to assess the performance of the main hydropneumatic spraying units used for phytosanitary treatment of fruit plants in Cuyo (Argentina). Measurements were taken at a standardized rate from the rear PTO (power take off) of the tractor. The air flow generated by the fan was assessed in a wind tunnel and the spatial distribution of air velocity was evaluated with a hot wire anemometer. The determination of vertical spray distribution was done with a lamellate patternator. Power demands were also determined. Air flows ranged between $257.49 \text{ m}^3 \cdot \text{min}^{-1}$ and $485.17 \text{ m}^3 \cdot \text{min}^{-1}$. Spraying units B and C showed asymmetric air distribution. Vertical spray distribution showed symmetry between the left and right sections, except for unit of equipment B in both working modes and E high. Power demand alternated between 11.96 kW and 31.17 kW. The most efficient units were A in its only available working mode D low and high and E low. Hydro pneumatic units have different aerodynamic capacities, both in released air flow and bilateral distribution and they have different power demands.

Keywords

air assisted sprayer • power • air flow rate • efficiency

INTRODUCCIÓN

El control de plagas en los cultivos constituye una preocupación constante para los agricultores, debido a la implicancia socioeconómica de la actividad, por un lado la agresión al medio ambiente y por otro la preocupación en disminuir las pérdidas y hacer rentable la producción (5). La protección fitosanitaria de los cultivos donde el objetivo a tratar es un volumen de vegetación se efectúa principalmente con equipos con doble circuito agua-aire (equipos hidroneumáticos) (2, 13, 19). El equipo pulverizador de ventilador axial es el diseño predominante en montes frutales por ser simple, robusto y comparativamente de bajo costo de compra y operación, además de ser efectivo en un amplio rango de tipos de frutales y de condiciones operativas (6).

La corriente de aire generada por el ventilador de un equipo de uso en frutales

juega un rol importante en el transporte y la penetración del pulverizado en la canopia del cultivo (9, 26). Los estudios de eficiencia de equipos de diferentes sistemas de aplicación han permitido corroborar las ventajas de aquellos de corriente de aire (9, 10).

Para conseguir un buen ajuste del equipo de tratamientos deben conocerse previamente: distribución de aire y líquido a diferentes alturas (20). Según Ade (1986), los principales elementos que caracterizan las prestaciones de un ventilador axial para máquinas pulverizadoras son: la cantidad de aire erogada en la unidad de tiempo, la velocidad de aire en correspondencia con las boquillas y la potencia absorbida a la toma posterior de potencia del tractor (TPP).

Algunos autores indican que las máquinas pulverizadoras con ventilador

axial generan distribuciones asimétricas de líquido (13, 28) y de aire (13, 17, 28) entre los sectores izquierdo y derecho de pulverización.

Debido a que las máquinas utilizadas en frutales cuentan con ventiladores de diferente tipo, con diámetros que oscilan entre 0,70 y 1,10 m, álabes con diferentes espesores, curvaturas y longitudes, en cantidad de 5 a 12 y con velocidades de regímenes variables, existen considerables diferencias en sus prestaciones (11). La gran variedad de marcas y modelos de máquinas pulverizadoras existentes, junto con la ausencia de adecuados manuales o guías de calibración son a menudo la causa de un escaso conocimiento técnico por parte del agricultor (7). Por la ausencia de estudios actuales en la región de Cuyo y el avance de la tecnología, resulta necesario realizar una evaluación de los equipos pulverizadores hidroneumáticos utilizados en montes frutales en dicha región para conocer su desempeño.

Hipótesis

Los equipos pulverizadores para frutales presentan simetría en la distribución bilateral de aire y de pulverizados.

Objetivos

Evaluar la velocidad y distribución de aire de los ventiladores.

Estudiar la distribución del pulverizado.

Verificar el requerimiento de potencia.

Determinar la eficiencia de cada equipo.

MATERIALES Y MÉTODOS

Para la evaluación comparativa se utilizaron equipos hidroneumáticos nuevos, comercializados en el año 2008,

de las principales empresas de máquinas pulverizadoras en la región de Cuyo, Argentina. Los ensayos se realizaron en un espacio exterior, sobre una superficie horizontal y lisa, para evitar las posibles alteraciones que pudieran producirse en un local cerrado.

Antes de iniciar los ensayos de medida de aire y de distribución vertical de la pulverización, se establecieron las condiciones de funcionamiento del equipo pulverizador a ensayar, fijando la orientación de las boquillas en el arco de distribución en forma simétrica y acorde a un árbol ideal de 4,5 m de altura, la orientación de los álabes del ventilador y la relación de multiplicación del ventilador. Los parámetros a medir fueron analizados a un régimen de trabajo de la TPP del tractor de $540 \text{ vueltas.min}^{-1}$ ($56,55 \text{ rad.s}^{-1}$) en la relación de transmisión alta y baja entre la TPP y el ventilador, si el equipo lo permitía. Para la evaluación de régimen de trabajo del tractor y del ventilador de la máquina pulverizadora se utilizó un tacómetro láser (Graigar, DT-2857, Shenzhen).

Las boquillas fueron operadas a una presión de $2 \cdot 10^6 \text{ Pa}$ (20 bar) que se corroboró mediante el manómetro de la máquina. Se utilizaron pastillas de cono hueco con un caudal nominal de 2880 ml.min^{-1} , según dato del fabricante. Para la evaluación del caudal real erogado por boquilla se cuantificó la descarga usando probetas conectados a cada pico con cañerías flexibles midiendo la cantidad de agua colectada después de un minuto. Las determinaciones se realizaron mientras funcionaban ambos arcos de pulverización y las mediciones comenzaron a realizarse después que la máquina llegó al régimen establecido (12).

La demanda de potencia de la TPP fue medido por medio de un torquímetro (420, Datum Electronics, Isle of Wight),

inserto en la unión cardánica entre la TPP y el eje de la bomba de la máquina pulverizadora. Las determinaciones se realizaron manteniendo la transmisión al ventilador y al agitador y con la bomba trabajando a la menor presión posible.

Para la evaluación de velocidad y caudal de aire se utilizó un túnel de viento construido según la norma IRAM 19004:1996, para ensayos de ventiladores. Para realizar determinaciones complementarias se utilizaron un tubo de Pitot (166-6-CF, Dwyer, Michigan), conectado a un manómetro digital con un rango de medición de 0 a 2,49 kPa (475-0-FM, Dwyer, Michigan), un psicrómetro y un barómetro (Skywatch Geos n° 11, JDC Instruments, Yverdon-les-Bains). El tubo de pitot se colocó paralelo al sentido de circulación del aire, en el centro del túnel y enfrentado al flujo de aire (15).

La distribución vertical del pulverizado fue cuantificada mediante un estudio de asperjado en banco de lamelas de 4500 mm de altura y 1600 mm de ancho de recepción de pulverizados. La altura total del banco está dividida en 18 cuerpos de recolección de diez lamelas cada uno (16).

La forma de las lamelas (deshumidificadores de aire) permite la retención del pulverizado y el posterior transporte favorecido por una pendiente del 5% respecto de la horizontal. Cada cuerpo recolecta el caldo fitosanitario y lo envía por desnivel a una probeta de 2000 cm³. Para la evaluación de la distribución vertical de pulverizados, se colocó el equipo hidroneumático a la mitad de la distancia entre hileras del cultivo de referencia (17), y se hizo funcionar durante noventa segundos. La distribución se expresó como porcentaje de líquido recolectado por sitio de censado.

La distribución de la velocidad del aire suministrada por el ventilador fue determinada con el equipo pulverizador fijo. Las determinaciones se realizaron con anemómetros ubicados a 1, 2, 3, 4 y 5 m de distancia del eje del ventilador. Estas mismas distancias se tomaron verticalmente desde el nivel del suelo. Se utilizó un anemómetro de hilo caliente (413-3, Dwyer, Michigan), cuya apertura fue dirigida hacia el centro del ventilador.

El equipo pulverizador fue colocado de tal forma que el aire fuera descargado paralelo a la estación de medición (24). Como el pulverizado puede tener inferencia en el funcionamiento del anemómetro, el líquido no fue pulverizado durante la evaluación de velocidad de aire.

La evaluación se realizó mediante la prueba de t apareada comparando los registros obtenidos correspondientes a sitios análogos de ambos sectores de aventado (izquierda y derecha) de cada equipo pulverizador

En la tabla 1 (pág. 347), se presentan las características de los equipos utilizados en los ensayos.

RESULTADOS Y DISCUSIÓN

El caudal de las boquillas de los equipos evaluados presenta una variación inferior a $\pm 5\%$ entre ambos sectores (tabla 2, pág. 348). En el 60% de los equipos evaluados existe al menos una boquilla que eroga caudales que exceden en $\pm 10\%$ los caudales indicados por el fabricante. En España se constató que dos de cada tres equipos evaluados presentaban igual heterogeneidad de pulverizado (25).

Tabla 1. Características de los equipos pulverizadores.

Table 1. Sprayers characteristics.

Equipo Pulverizador	Diámetro de ventilador (mm)	Nº de álabes	Longitud de álabes (mm)	Área de toma de aire (m ²)	Área de salida de aire total (m ²)
A	850	10	260	0,608	0,403
B baja	800	8	300	0,528	0,273
B alta					
C baja	800	7	200	0,442	0,212
C alta					
D baja	740	8	270	0,478	0,315
D alta					
E baja	830	8	230	0,694	0,336
E alta					

Equipo Pulverizador	Presencia de deflectores móviles	Nº de boquillas	Relación de transmisión entre toma de fuerza y ventilador	Régimen de ventilador (vueltas.min ⁻¹)
A	Si	18	1:3,16	1708
B baja	No	14	1:3,46	1870
B alta			1:4,55	2457
C baja	No	14	1:3,87	2092
C alta			1:4,94	2667
D baja	No	14	1:3,70	2000
D alta			1:4,53	2444
E baja	Si	16	1:2,95	1591
E alta			1:3,84	2071

En la figura 1a (pág. 348), se presentan los resultados correspondientes a la evaluación de caudal de aire de los equipos. Se representan los valores medios y el error estándar para cada uno de los equipos. El equipo C baja y el equipo E baja son los que menor caudal de aire erogan ($257,49 \pm 8,42$ y $266,21 \pm 4,91$ m³.min⁻¹, respectivamente), mientras que el equipo A es el que eroga un mayor caudal de aire ($485,17 \pm 6,30$ m³.min⁻¹).

Balsari y Oggero (2001) encontraron caudales entre 367 y 667 m³.min⁻¹

(a 500 vueltas.min⁻¹ de la TPP), valores superiores a los obtenidos con los equipos B baja, C baja, D baja, E baja y C alta.

Estos caudales obtenidos se encuentran dentro del rango de caudales de aire encontrados en España que varían entre 200 m³.min⁻¹ y 833 m³.min⁻¹ (23). Pezzi y Rondelli (2000), ensayando un equipo pulverizador con un ventilador de 600 mm, obtuvieron una generación de aire de 432 m³.min⁻¹ con una velocidad de giro de 500 vueltas.min⁻¹ de la TPP.

Tabla 2. Caudales promedio de líquido erogado y su variación.
Table 2. Mean liquid flow rate and his variation.

Equipo	Caudalimetría (ml.min ⁻¹)		Variación (%) ^(*)	Coeficiente de Variación % ^(**)		Boquillas que exceden ±10% el caudal nominal ^(**)	
	Sectores de Asperjado			Izquierdo	Derecho	N°	%
	Izquierdo	Derecho					
A	2875,56	2758,52	4,07	4,43	3,25	0	0,0
B	2722,86	2853,33	4,57	14,42	1,21	1	7,1
C	2740,95	2841,90	3,55	2,63	5,65	1	7,1
D	2916,19	2888,57	0,95	2,06	2,26	0	0,0
E	2675,00	2741,67	2,43	3,52	3,41	2	12,5

(*) Variación (%) = (a-b).100/a; siendo a el valor de caudalimetría mayor y b el valor menor, para cada accionamiento.

(**) Caudal nominal: 2880 ml.min⁻¹.

(*) Variation (%) = (a-b).100/a; being a the highest value and b the lowest value, for each working mode.

(**) Nominal flow: 2880 ml.min⁻¹.

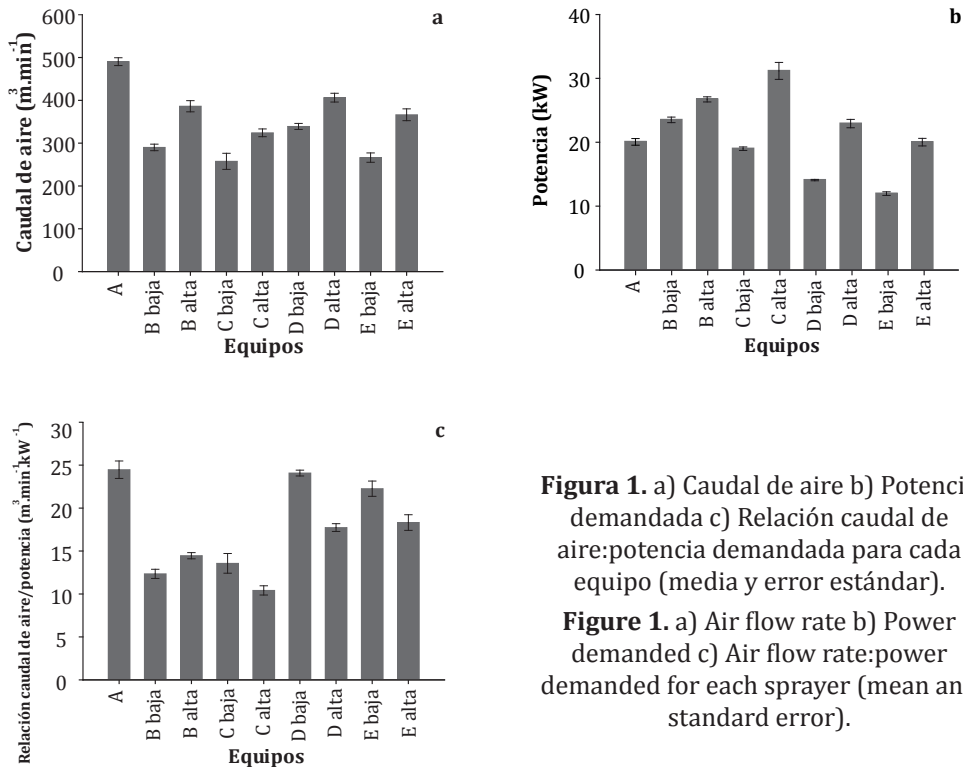


Figura 1. a) Caudal de aire b) Potencia demandada c) Relación caudal de aire:potencia demandada para cada equipo (media y error estándar).

Figure 1. a) Air flow rate b) Power demanded c) Air flow rate:power demanded for each sprayer (mean and standard error).

Considerando los ensayos locales, el rango de valores encontrado en este ensayo es similar al hallado por Del Monte *et al.* (datos no publicados) desde $300 \text{ m}^3 \cdot \text{min}^{-1}$ a $700 \text{ m}^3 \cdot \text{min}^{-1}$ para equipos utilizados en fruticultura. Por el contrario, los caudales son inferiores a los presentados por Di Prinzio (1989) quien indica valores entre $593 \text{ m}^3 \cdot \text{min}^{-1}$ y $719 \text{ m}^3 \cdot \text{min}^{-1}$.

Considerando el intervalo de caudales de aire entre $330 \text{ m}^3 \cdot \text{min}^{-1}$ y $500 \text{ m}^3 \cdot \text{min}^{-1}$ propuesto como óptimo por Balsari *et al.* (2007) para frutales, existen algunos equipos que se ubican fuera del rango por presentar valores inferiores (B baja, C baja, C alta y E baja). Estos equipos no serían adecuados para la realización de aplicaciones fitosanitarias.

En la figura 1b (pág. 348), se presentan los resultados correspondientes al análisis de potencia demandada (media y error estándar).

El equipo E baja es el que menor potencia demanda ($11,96 \pm 0,13 \text{ kW}$), mientras que el equipo C alta es el más demandante de potencia ($31,17 \pm 0,59 \text{ kW}$). Se observa una diferencia de aproximadamente 20 kW entre los equipos menos demandantes de potencia (E baja y D baja) y el equipo C alta. La potencia demandada se encuentra entre los valores hallados por Balsari y Oggero (2001), quienes obtienen valores entre $5,97$ y $34,3 \text{ kW}$ (a $500 \text{ vueltas} \cdot \text{min}^{-1}$ de la TPP) y, mayoritariamente, con los resultados hallados por Planas y Fillat (1991) en España, quienes presentan valores entre $14,9$ y $35,05 \text{ kW}$.

En la figura 1c (pág. 348), se presentan los resultados correspondientes a la relación entre caudal de aire y potencia demandada. Se grafican los valores medios y el error estándar para cada uno de los equipos.

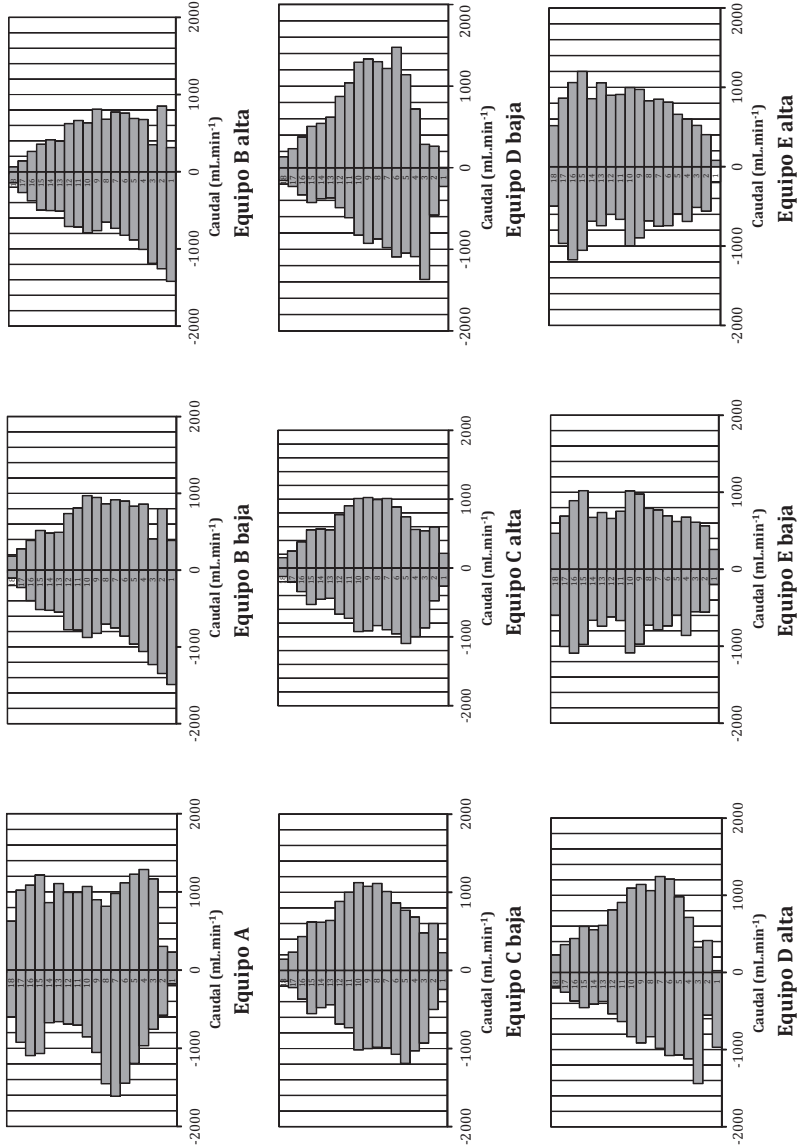
Los equipos que más eficientemente aprovechan la potencia para generar las corrientes de aire son A, D baja y E baja ($24,17 \pm 0,49 \text{ m}^3 \cdot \text{min}^{-1} \cdot \text{kW}^{-1}$, $24,08 \pm 0,15 \text{ m}^3 \cdot \text{min}^{-1} \cdot \text{kW}^{-1}$ y $22,26 \pm 0,40 \text{ m}^3 \cdot \text{min}^{-1} \cdot \text{kW}^{-1}$, respectivamente), mientras que los equipos C alta y B baja son los que menos eficientemente utilizan la potencia absorbida ($10,41 \pm 0,24 \text{ m}^3 \cdot \text{min}^{-1} \cdot \text{kW}^{-1}$ y $12,35 \pm 0,24 \text{ m}^3 \cdot \text{min}^{-1} \cdot \text{kW}^{-1}$, respectivamente).

En la figura 2 (pág. 350), se presentan los gráficos sobre resultados de recolección de pulverizados en altura en banco de lamelas para todos los equipos evaluados y los regímenes establecidos.

La prueba de t para datos apareados indica que los equipos B en sus dos accionamientos y E alta presentan asimetría significativa ($\alpha \leq 0,05$) en su distribución vertical de pulverizados, para ambos sectores de recepción (tabla 3, pág. 351). Coincidiendo con estos resultados, Giles *et al.* (1989) y Vujčić *et al.* (2015), indican que las máquinas pulverizadoras con ventiladores axiales generan distribuciones asimétricas de líquido.

Los resultados obtenidos con los equipos evaluados demuestran gran variabilidad respecto de los sectores con mayor recolección de líquido.

Travis *et al.* (1987) trabajando con manzanos medianos y pequeños de canopias densas y Magdalena *et al.* (1996) utilizando diferentes máquinas pulverizadoras en perales, obtuvieron menores recolecciones en la zona alta. En ensayos de laboratorio con banco de lamelas, Holownicki *et al.* (2000), encontraron la misma tendencia de valores más bajos en la zona alta con diferentes máquinas pulverizadoras; estos resultados concuerdan con los obtenidos con los equipos B, C y D.



(*) Los valores del sector izquierdo son presentados con signo negativo para favorecer la visualización.

(*) The left values are presented with a negative sign to favor visualization.

Figura 2. Distribución vertical de pulverizado en banco de lamelas de cada equipo a distintos accionamientos de la TPP (*).

Figure 2. Vertical spray distribution in lamellae patternator of each sprayer at different drives of the PTO (*).

Tabla 3. Eficiencia de pulverizadores hidroneumáticos.

Table 3. Efficiency of air assisted sprayer.

Equipo	Relación caudal de aire/potencia	Distribución bilateral hidroneumática	
		Simetría de distribución de aire (*)	Simetría de distribución de pulverizado (*)
A	24,17	Sí (p=0,2938)	Sí (p=0,4750)
B baja	12,35	No (p<0,0001)	No (p=0,0053)
B alta	14,46	No (p<0,0001)	No (p<0,0001)
C baja	13,56	No (p<0,0001)	Sí (p=0,8999)
C alta	10,41	No (p<0,0001)	Sí (p=0,9618)
D baja	24,08	Sí (p=0,7075)	Sí (p=0,1117)
D alta	17,73	Sí (p=0,9429)	Sí (p=0,7199)
E baja	22,26	Sí (p=0,7439)	Sí (p=0,1055)
E alta	18,32	Sí (p=0,5654)	No (p=0,0124)

(*) Simetría = diferencia significativa para prueba de t apareada, $\alpha \leq 0,05$ entre los sectores izquierdo y derecho. Entre paréntesis se presentan valores de p.

(*) Symmetry = significant difference for paired t test, $\alpha \leq 0.05$ between the left and right sectors. In parentheses p-values are presented.

Cunningham y Harden (1998), encontraron menores depósitos en la zona central y mayores en la zona baja, pulverizando mandarinos con volúmenes de caldo de pulverización entre 1000 y 8000 L.ha⁻¹. Este perfil de distribución de pulverizados no coincide con ninguno de los equipos ensayados.

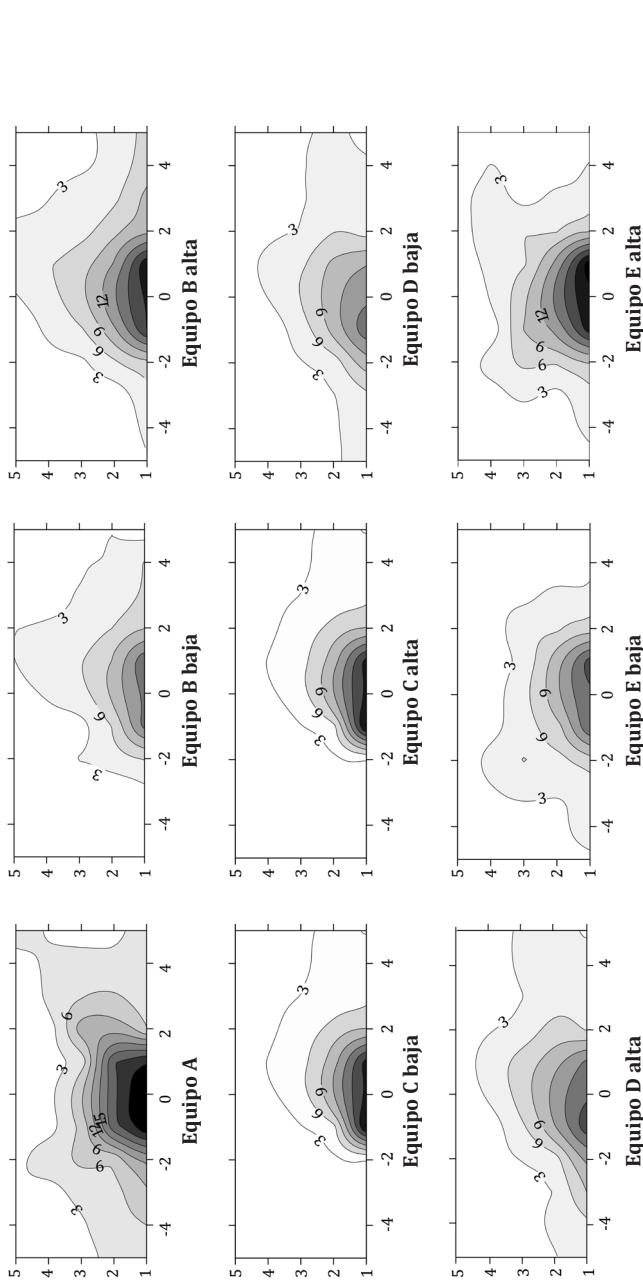
En la figura 3 (pág. 352), se presentan los perfiles anemométricos de los diferentes equipos evaluados. Los patrones de distribución de aire para cada equipo en particular utilizando diferentes accionamientos son similares entre sí. Los mismos resultados fueron encontrados por Pergher y Petris (2008), utilizando una máquina pulverizadora para viñedos.

En la tabla 3 se presentan los datos de evaluación de la velocidad de aire.

Los equipos que presentan diferencias estadísticas significativas entre ambos sectores son B y C en todos sus accionamientos; Magdalena (2003), también encuentra asimetría entre los sectores izquierdo y derecho del ventilador de una máquina pulverizadora de frutales; esta misma asimetría es descrita por Giles *et al.* (1989) y por Vujčić *et al.* (2015), para máquinas pulverizadoras con ventiladores axiales.

Para evaluar la eficiencia de los equipos se tuvo en cuenta la variabilidad de la distribución del aire y del líquido a ambos lados del equipo pulverizador y la potencia requerida para el movimiento de una masa de aire (considerando el umbral en 15 m³.min⁻¹.kW⁻¹).

En la tabla 3 se presentan los valores de eficiencia del ventilador. Los equipos de mayor eficiencia son A, D baja, D alta y E baja.



(*) Los valores colocados sobre las líneas indican velocidad de aire en $m.s^{-1}$. Los valores del sector izquierdo son presentados con signo negativo para favorecer la visualización.

(*) The values above the lines indicate air velocity in $m.s^{-1}$. The values of the left sector are presented as negative to favor the visualization.

Figura 3. Perfil anemométrico de cada equipo a distintos accionamientos de la TPP (*).

Figure 3. Anemometer profile of each sprayer at different drives of the PTO (*).

CONCLUSIONES

La existencia de diferentes fabricantes de pulverizadores genera equipos con características constructivas diversas y desiguales rendimientos.

Los equipos presentan diferentes capacidades aerodinámicas, tanto en caudal de aire erogado como en su distribución bilateral. Sin embargo, la distribución simétrica de la corriente de aire no produce siempre una distribución de pulverizados simétrica.

El conocimiento de la distribución de líquido en altura permite la elección del equipo más adecuado a la geometría del cultivo a tratar y/o regular el caudal de las boquillas y la deflexión de aire.

Los equipos pulverizadores difieren en la demanda de potencia como también en su eficiencia para generar aire.

El conocimiento de los parámetros aerodinámicos y de distribución de los pulverizados de una atomizadora, puede ser de utilidad para que los fabricantes mejoren sus diseños con el objetivo de aumentar la eficiencia de las aplicaciones fitosanitarias.

Sería conveniente que los fabricantes de pulverizadoras entregaran información técnica respecto de caudales de aire y distribución de aire y pulverizado de los equipos comercializados, para que los usuarios puedan disponer de herramientas para seleccionar el equipo que más se adapte a su cultivo.

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Control of *Rhizoctonia solani* with extracts from *Ovidia andina*

Control de *Rhizoctonia solani* con extractos de *Ovidia andina*

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ABSTRACT

New alternatives are needed to control ubiquitous soilborne fungi like *Rhizoctonia solani*. This work evaluates the activity of extracts from *Ovidia andina* on this pathogen. Powdered dried leaves and stems were extracted by maceration with dichloromethane (CH₂Cl₂), methanol and ethanol. An aqueous extract was obtained by decoction, and the hydrodistillation residual water was the aqueous phase after essences production. A strain of *R. solani* was cultivated on potato dextrose agar supplemented with the extracts, at 1:100 and 1:1000 v/v. The CH₂Cl₂ extract at 1:100 reduced colony growth (p< 0.0001) and changed hyphal morphology. Five fractions were obtained from the CH₂Cl₂ extract in a glass column, and TLC analysis showed the presence of coumarins and flavonoids. The CH₂Cl₂ extract and fractions were diluted in CH₂Cl₂ or acetone. The efficiency to reduce pathogen growth of fractions 2f and 5f diluted in CH₂Cl₂ at 1:100 did not differ from the whole extract (p<0.0001). For an *in vivo* test, beetroot seed balls were incubated in soil infested with *R. solani* and treated by immersion in the CH₂Cl₂ extract at 1:100 for different periods of time. As a result, pathogen colonization was diminished by immersion for 120 s and seed germination was normal (p< 0.0001). Additionally, infested soil was treated with the CH₂Cl₂ extract, and inoculum concentration estimated using beetroot seed balls as baits diminished (p< 0.0001). These results lead us to conclude that *O. andina* is a source of antifungal components for crop protection against *R. solani*.

Keywords

active fractions • bioguided fractionation • CH₂Cl₂ extract • coumarins and flavonoids characterization • fungal growth

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RESUMEN

Resulta necesario generar alternativas para el control de hongos patógenos de suelo como *Rhizoctonia solani*. En este trabajo, se evaluó la actividad de extractos de *Ovidia andina* sobre una cepa del patógeno. Se realizaron extracciones a partir de hojas y tallos secos pulverizados, macerados con diclorometano (CH_2Cl_2), metanol y etanol. Se obtuvo un extracto acuoso por decocción y aguas residuales consistentes en la fase acuosa luego de la producción de esencias. Se cultivó una cepa de *R. solani* en agar papa glucosa suplementado con los extractos diluidos 1:100 o 1:1000 v/v. El extracto CH_2Cl_2 1:100 redujo el crecimiento de las colonias ($p < 0,0001$) y alteró la morfología de las hifas. Se obtuvieron 5 fracciones del extracto CH_2Cl_2 en una columna de vidrio y mediante TLC se detectó la presencia de cumarinas y flavonoides. Posteriormente, el extracto CH_2Cl_2 y sus fracciones se diluyeron en CH_2Cl_2 o acetona. La eficiencia de las fracciones 2f y 5f diluidas CH_2Cl_2 1:100 para reducir el crecimiento fúngico no difirió de la del extracto completo ($p < 0,0001$). En un experimento *in vivo*, se incubaron glomérulos de remolacha en suelo infestado con *R. solani* y se sumergieron en el extracto CH_2Cl_2 1:100 durante tiempos variables. La colonización fúngica disminuyó mediante la inmersión durante 120 segundos, sin afectar la germinación ($p < 0,0001$). Adicionalmente, se trató suelo infestado con el extracto CH_2Cl_2 y disminuyó la concentración de inóculo del patógeno estimada utilizando glomérulos de remolacha como trampas ($p < 0,0001$). Estos resultados permiten concluir que *O. andina* es una fuente de componentes antifúngicos para la protección vegetal contra *R. solani*.

Palabras clave

fracciones activas • fraccionamiento bioguiado • extracto CH_2Cl_2 • caracterización de cumarinas y flavonoides • crecimiento fúngico

INTRODUCTION

Rhizoctonia solani J. G. Kühn is a causative agent of seedling damping-off and crown and root rot diseases, with a total of 2,593 fungus-host combinations recorded around the world (10). This species has been identified on a wide range of hosts that includes vegetables, oil crops, forest trees, forages, ornamentals, fruits, and cereals (21). Pathogenic strains of *R. solani* may be transmitted by soil and also by seeds of many hosts (27). Although the most important control measures are cultural, chemicals are still important tools to reduce the damages caused by *R. solani* (32). However, the concerns about the

safety and environmental impact of chemicals have focused research on alternative strategies to manage plant diseases (26).

Plants constitute a potential source of chemical structures, especially secondary metabolites (1, 13) that could be appropriate for crop protection, in an integrated management framework (3). The shrub *Ovidia andina* (Poepp. and Endl.) Meisn. belongs to the Thymelaeaceae and is commonly known as pillo-pillo (36) or traro-voqui (30). This species occurs from 700 to 2,300 m elevation in central Chile and along the adjacent Andean slopes of Argentina (19).

The Mapuche community uses it as a medicinal plant (17). As many other native species, *O. andina* may be of interest to be evaluated for plant protection. The objectives of this work were to obtain extracts and purified fractions from *O. andina*, to evaluate their efficiency to control *R. solani* and to characterize the bioactive substances.

MATERIALS AND METHODS

Plants and pathogen

O. andina plants were collected at Cerro Centinela (43°29'0" S 71°35'53" W), located in Esquel, province of Chubut, Argentina in May. An entry coded as BAF 17501 was deposited at the Herbarium of the Museo de Farmacobotánica J. A. Domínguez, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. The plant material was collected by Dr. Silvia González (Universidad Nacional de la Patagonia San Juan Bosco, Esquel), and the identification was confirmed by Dr. Nidia Hansen (INTA, Esquel).

The pathogenic isolate O-IM-1, belonging to *Rhizoctonia solani* AG-4 HG-II and isolated in Buenos Aires (25) is part of a fungal collection of the Cátedra de Fitopatología, Facultad de Agronomía, Universidad de Buenos Aires. It was maintained on potato dextrose agar (PDA, Merck) and used throughout all this study. Beetroot (*Beta vulgaris* L., Amaranthaceae) was chosen as bait to estimate inoculum density of *R. solani*, as well as target for disease control assays. Beetroot fruits are the organs used to initiate the crop and are commonly referred to as seeds or seed balls.

Extracts and extract characterization

Plants of *O. andina* were dried by forced air at room temperature, and leaves and stems were ground with a blade mill.

The powder (100 g) was soaked in dichloromethane (CH₂Cl₂) over a 24 h period, and filtered through Whatman No. 1 filter paper (gravity filtration). The protocol was repeated three times, and then the CH₂Cl₂ extracts were pooled and taken to dryness in a rotary evaporator. The plant drug previously extracted with CH₂Cl₂ was air dried over a 24 h period, soaked in methanol for 24 h, and finally filtered using Whatman No. 1 filter paper (gravity filtration). This was repeated three times. Subsequently, the methanolic (MeOH) extracts were pooled, dried in a rotary evaporator and lyophilized.

A 70% v/v ethanolic (EtOH) extract was obtained as follows: 12 g of powdered and dried aerial organs were placed in an Erlenmeyer flask, extracted with 400 mL of 70% v/v ethanol under agitation with a magnetic stir bar for 30 min, and filtered through Whatman No. 1 filter paper. This procedure was repeated three times. The extracts were pooled, dried in a rotary evaporator and lyophilized.

An aqueous (Aq) extract was obtained by boiling 20 g of powdered and dried leaves and stems in an Erlenmeyer flask containing 400 mL of distilled water for 20 min. The decoction was allowed to cool to 40-45°C, filtered through a Whatman No. 1 filter paper (gravity filtration) and the volume restored to 400 mL by adding distilled water (9), and lyophilized (FTS Flexi-Dry lyophilizer).

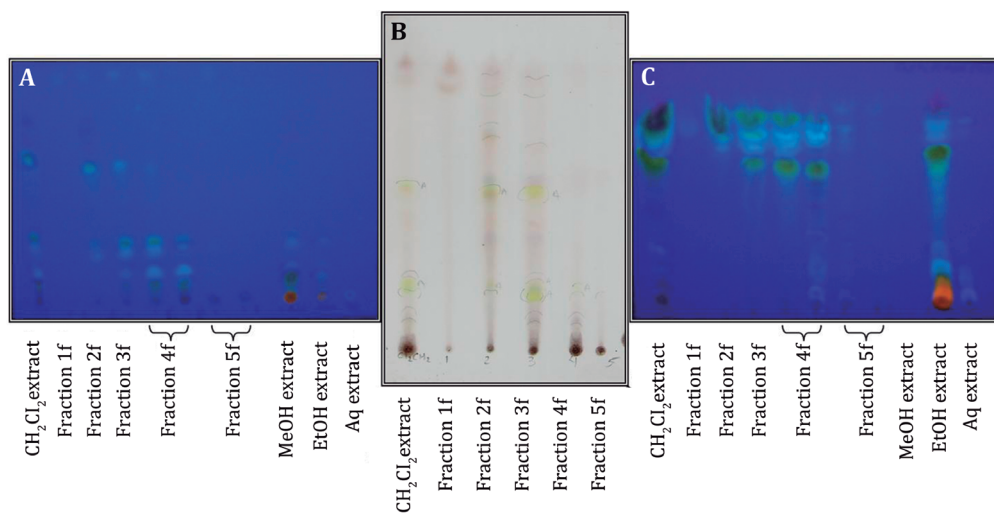
Additionally, the aqueous phase that remained after hydrodistillation to obtain essences from *O. andina* (9) was filtered, reduced in volume in a rotary evaporator, lyophilized and named hydrodistillation residual water (Hrw).

After analysis by means of the first bioassay (test 1) described later, two other assays (tests 2 and 3) were employed to quantify the activities of the CH₂Cl₂ extract fractions.

The extract (90 mg) was fractionated at atmospheric pressure in an open glass column (4 cm x 3.5 cm), employing Macherey-Nagel silica gel 60 (0.063-0.2 mm / 70-230 mesh ASTM for column chromatography) as stationary phase, and solvents or solvent mixtures of increasing polarity as mobile phases (toluene, toluene + ethyl ether (7:3), toluene + ethyl ether (1:1), ethyl ether, ethyl ether + methanol (1:1), methanol).

Fractioning was repeated four times, and the fractions eluted through the columns were analyzed by thin layer chromatography (TLC). Similar fractions from the four columns were collected, obtaining seven fractions (1 to 7). All the extracts and fractions were analyzed

through the following TLC systems: I) Stationary phase (SP): silica gel, Mobile phase (MP): toluene:ethylacetate (8:2); II) SP: silica gel; MP: CH₂Cl₂:MeOH (9:1). The chromatograms were developed with Natural Product reagent (NP) Fluka, a polyphenol reagent, and observed under natural and UV 366 nm light (TLC systems I and II) (figure 1A, figure 1C). The fractions 4 and 5 as well as the fractions 6 and 7, with similar profiles in both systems were pooled in the final fractions 4f and 5f respectively. This fractionation was guided according to antifungal activity. The final fractions were named 1f to 5f. The TLC system I was also developed with 50% v/v H₂SO₄, a universal reagent, and observed under natural light (figure 1B) (33).



CH₂Cl₂: dichloromethane extract, MeOH: methanolic extract, EtOH: ethanolic extract, Aq: aqueous extract, Fractions 1f to 5f: final fractions of the CH₂Cl₂ extract.

CH₂Cl₂: extracto diclorometánico, MeOH: extracto metanólico, EtOH: extracto etanólico, Aq: extracto acuoso, Fracciones 1f a 5f: fracciones finales del extracto CH₂Cl₂.

Figure 1. Thin layer chromatography (TLC) of the extracts and fractions obtained from *Ovidia andina*. A. TLC system I, developed with natural products (NP) reagent. B. TLC system I, developed with sulfuric acid - ethanol 50% v/v reagent. C. TLC system II, developed with NP reagent.

Figura 1. Cromatografía en capa fina (TLC) de los extractos y fracciones obtenidos de *Ovidia andina*. A. sistema TLC I revelado con reactivo NP. B. sistema TLC I revelado con ácido sulfúrico - etanol 50% v/v. C. sistema TLC II revelado con reactivo NP.

Pathogen control

Colony growth

Three assays (tests 1 to 3) were performed. In test 1, *R. solani* strain O-IM-1 was grown on PDA supplemented with different extracts obtained from *O. andina*. For that purpose, the CH₂Cl₂ extract was diluted in CH₂Cl₂; the MeOH, EtOH and Aq extracts were diluted in 80% v/v ethanol, and the HrW was diluted in water, all at 1:10 and 1:100 v/v (controls: CH₂Cl₂, ethanol, water). One mL of each dilution was pipetted into a Petri dish, and all the plates were kept open under laminar flow, time enough to allow the evaporation of the solvent and consequently avoid its interference (8). Ten mL of molten PDA were then poured into each plate, so that final extract dilutions were 1:100 and 1:1000. Plugs of 15 mm diameter were cut from the edge of colonies of *R. solani* of 5 days on PDA and used as a source of inoculum, and one plug was placed in the center of each plate after the PDA had solidified. The plates were randomly distributed in an incubator, at 22°C. Three replicates were done per treatment and the experiment was conducted three times. Pathogen radial growth was registered daily and the inhibition was calculated (31). Pathogen hyphae were observed with a compound microscope.

For test 2, the pathogen was grown on PDA supplemented with the CH₂Cl₂ extract and fractions 1f to 5f diluted in CH₂Cl₂ at 1:100 (control: CH₂Cl₂). For test 3, the pathogen was grown on PDA supplemented with the CH₂Cl₂ extract and fractions 1f to 5f diluted in acetone at 1:100 (control: acetone). The protocol for tests 2 and 3 was identical to the one detailed for test 1.

Seed ball colonization and seed germination

A sample of field soil was placed in 4.4 dm³ metal containers, autoclaved at 1 atm for 1 h and allowed to cool. Plugs

of 1 cm³ from the edges of a colony of *R. solani* on PDA were placed on the surface of the soil (0.1% v/v), the containers were sealed with aluminum foil and incubated at room temperature over a period of 5 days (25) so as to obtain inoculum for the experiments described below. Groups of fifty beetroot seed balls were evenly distributed over 32 g of the infested soil in a Petri dish, and covered with an additional 32 g of infested soil. After 48-h incubation at 26°C, the seed balls were recovered, washed for 5 min under running tap water in a colander, and excess water was blotted with paper towels. Samples of 10 fungal-exposed seed balls were immersed into 2 mL of the CH₂Cl₂ extract 1:100 diluted in CH₂Cl₂ (controls CH₂Cl₂, water) contained in test tubes and shaken with a vortex shaker. Three replications were done per treatment and the assay was repeated twice. Immersion times were 30, 60, 90, 120 and 150 s. The seed balls were plated on PDA (pH4) (16) placing 50 seed balls in each plate. The number of seed balls with emerging hyphae of *R. solani* was determined by observations under a stereomicroscope after 24 h. Each seed ball with emerging *R. solani* hyphae was counted as positive. Colonies of *R. solani* that developed from beetroot seed balls were photographed 24 h after evaluation under the stereo microscope. A previous assay was done to verify that *R. solani* was not present in the seed balls before the treatments, by a modification of Ko and Hora (16) method: sterilized soil was used instead of colonized soil, and the seed balls were immersed in water for 30, 60, 92, 120 and 150 s. As described above, the seed balls were plated on PDA and the number of seed balls with emerging hyphae of *R. solani* was determined by observations under the stereomicroscope after 24 h.

Another assay was done to verify absence of dormancy in the seeds (results not shown), so as to determine the need of a 2 h washing treatment to eliminate water soluble germination inhibitors located in the pericarp. A germination test was followed in order to evaluate the effect of the CH₂Cl₂ extract on beetroot. Seed balls were immersed for 120 s in the CH₂Cl₂ extract 1:100 (controls: CH₂Cl₂, water), placed on moistened, pleated sheets of tissue paper and incubated at 20°C (7). Five replicates were done per treatment and the experiment was conducted three times. Percentage of germination was evaluated at 14 and 21 days.

Soil infestation

A sample of field soil was infested with the strain of *R. solani* and placed in Petri dishes, following the method already described. Beetroot seed balls were placed in the middle of the two layers of 32 g of soil, as baits for a further estimation of the inoculum concentration of *R. solani* in the soil treated with the CH₂Cl₂ extract 1:100. One mL of the CH₂Cl₂ extract was pipetted into each Petri dish just before placing the soil (controls: CH₂Cl₂, water). Five replications were done per treatment and the assay was repeated twice. After 48 h of incubation at 26°C, the seed balls were recovered, washed and incubated on PDA (10 seed balls plate⁻¹) before observation

under the stereomicroscope. Each seed ball with emerging *R. solani* hyphae was counted as positive. Inoculum concentration in the soil was estimated as the mean number of fungal propagules gram⁻¹ of soil (16).

Statistical analysis

All the tests were designed at random. Infostat software (FCA-UNC, Córdoba) was used for statistical calculations. Split-plot methodology was chosen for two-factor ANOVAs when time was one of the factors; and Satterwaite procedure was used for multiple comparisons when interactions between factors were significant (seed germination, pathogen growth). DGC test was chosen for one-factor analyses (pathogen growth), while Kruskal Wallis non parametric test was used for the analysis of seed ball colonization.

RESULTS

Extracts and extract characterization

Table 1 summarizes the quantities of extracts obtained from leaves and stems of *O. andina*, and their respective yields (g extract 100g⁻¹ leaves and stems). The TLC analysis of the CH₂Cl₂, MeOH, EtOH and Aq extracts and the fractions 2f to 5f of the CH₂Cl₂ extract showed the presence of coumarins and flavonoids (figure 1, page 358).

Table 1. Extracts obtained from the samples of *Ovidia andina* with different solvents.

Tabla 1. Extractos obtenidos de las muestras de *Ovidia andina* con solventes diferentes.

Extract	Leaves + stems (g)	Extract (g)	Yield ¹ (%)
Cl ₂ CH ₂ (dichloromethanic)	100.0	4.0	4.0
MEOH (methanolic) ²	100.0	7.4	7.4
EOH (ethanolic)	12.2	2.1	17.2
Aq (aqueous)	20.1	2.9	14.4

¹g extract 100g⁻¹ leaves and stems. ²after extraction with CH₂Cl₂.

¹g extracto 100g⁻¹ hojas y tallos. ²luego de la extracción con CH₂Cl₂.

The coumarins were observed as light blue spots and the flavonoids as yellow spots when treated with NP reagent and viewed under UV light at 366 nm, and the flavonoids were observed as yellow spots when revealed with 50% H₂SO₄ reagent.

Pathogen control

Colony growth

Interactions between treatments and times of evaluation were significant ($p < 0.0001$) in the three tests.

Figure 2 shows the results of multiple comparison tests for the growth of *R. solani* at the end of test 1.

The CH₂Cl₂ extract at 1:100 inhibited fungal growth, and the rest of the treatments did not differ from the control.

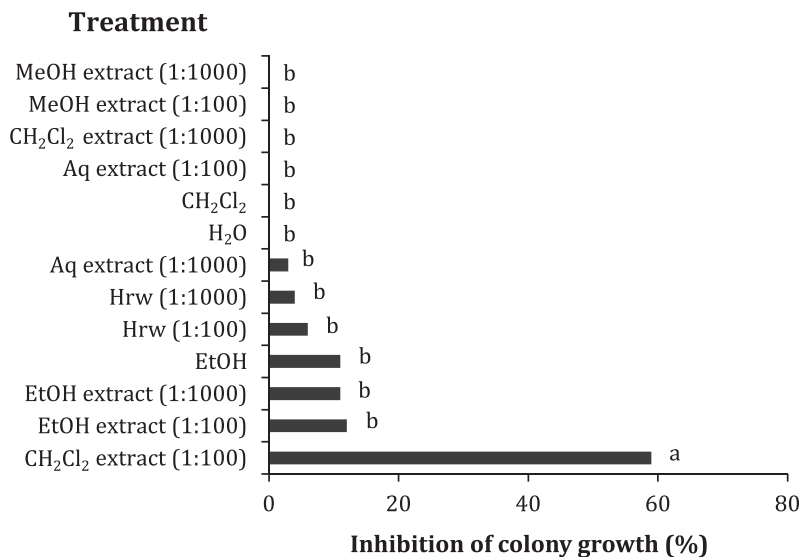
Hyphae appeared widened, swollen and contained discrete granules following treatments with the CH₂Cl₂ extract at 1:100 (figure 3, page 362).

In test 2, the best efficiency was observed for the CH₂Cl₂ extract, fraction 2f and fraction 5f; followed by fractions 3f and 1f; all diluted in CH₂Cl₂ (figure 4, page 362).

In test 3, the best efficiency was observed for the CH₂Cl₂ extract, followed by fraction 2f and fraction 3f; all diluted in acetone (figure 5, page 363).

Seed ball colonization and seed germination

The sample of beetroot seed balls tested for the presence of the pathogen showed no evidence of *R. solani* previous to the seed ball colonization test.

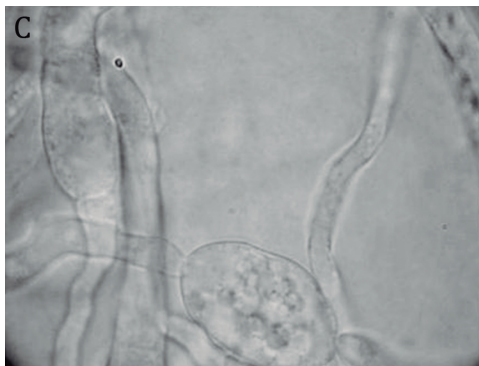


Different letters indicate differences between treatments. DGC test, α : 0.05.

Letras diferentes indican diferencias entre tratamientos. Prueba DGC, α : 0,05.

Figure 2. Growth of *R. solani* on potato dextrose agar supplemented with the extracts obtained from *Ovidia andina*, diluted in CH₂Cl₂.

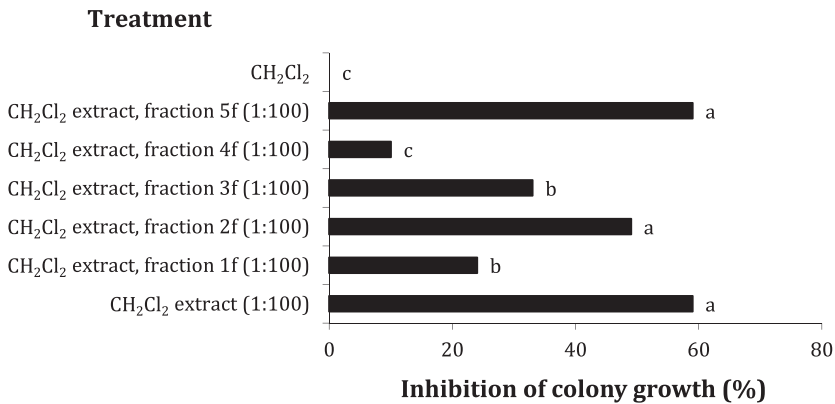
Figura 2. Crecimiento de *R. solani* en agar papa glucosa suplementado con los extractos de *Ovidia andina* diluidos en CH₂Cl₂.



A. Control. B. Widened hypha and granules. C. Swollen hyphae.
 A. Testigo. B. Hifa ensanchada y presencia de gránulos. C. Hifa hinchada.

Figure 3. Microscope observations of hyphae of *R. solani* after treatments with the dichloromethanic (CH_2Cl_2) extract of *Ovidia andina*.

Figura 3. Observaciones al microscopio de hifas de *R. solani* luego del tratamiento con el extracto diclorometánico (CH_2Cl_2) de *Ovidia andina*.

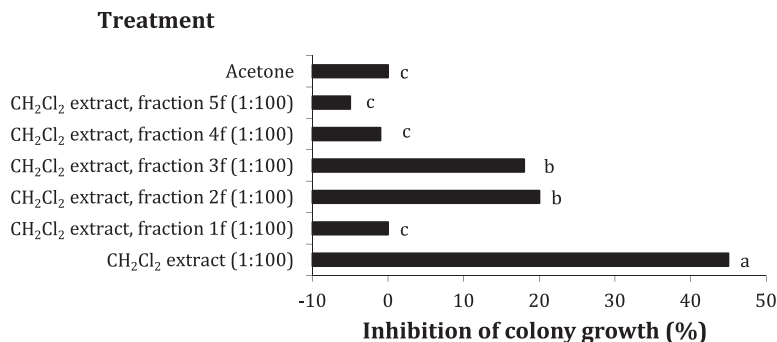


Different letters indicate differences between treatments. DGC test, α : 0.05.

Letras diferentes indican diferencias significativas entre tratamientos. Prueba DGC, α : 0,05.

Figure 4. Growth of *Rhizoctonia solani* on PDA supplemented with the CH_2Cl_2 extract from *Ovidia andina* and its fractions, diluted in CH_2Cl_2 .

Figura 4. Crecimiento de *Rhizoctonia solani* en agar papa glucosa suplementado con el extracto CH_2Cl_2 de *Ovidia andina* y sus fracciones, diluidas en CH_2Cl_2 .



Different letters indicate differences between treatments. DGC test, α : 0.05.

Letras diferentes indican diferencias significativas entre tratamientos. Prueba DGC, α : 0,05.

Figure 5. Growth of *Rhizoctonia solani* on potato dextrose agar supplemented with the CH₂Cl₂ extract from *Ovidia andina* and its fractions, diluted in acetone.

Figura 5. Crecimiento de *Rhizoctonia solani* en agar papa glucosa suplementado con el extracto CH₂Cl₂ de *Ovidia andina* y sus fracciones diluidos en acetona.

Levels of colonization by *R. solani* were significantly diminished following immersion in the CH₂Cl₂ extract diluted in CH₂Cl₂ for 120 and 150 s (figure 6, page 364).

Beetroot germination was delayed by immersion in the CH₂Cl₂ extract, as it was reduced at day 14, but similar to the control at day 21 (table 2, page 364). Seedlings developed from seed balls treated with CH₂Cl₂ were not visibly different from the control.

Soil infestation

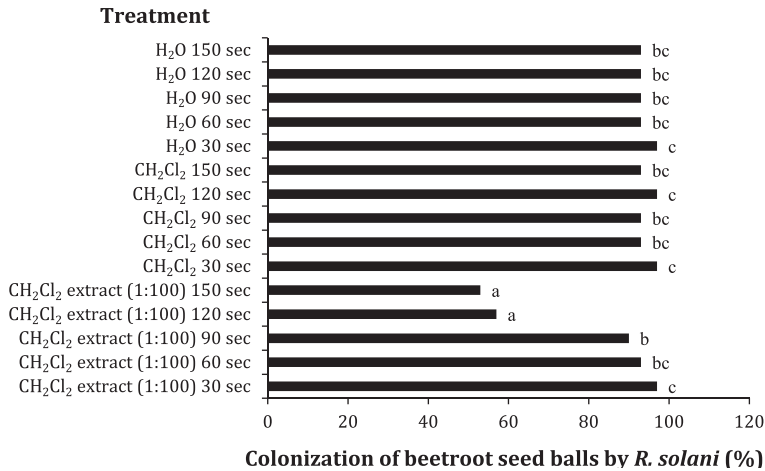
Inoculum density of *R. solani* estimated as the number of propagules in the soil diminished in the extract treatments (table 2, page 364). Figure 7 (page 365) shows the colonies of *R. solani* that developed on PDA from beetroot seeds 24 h after evaluation under the stereomicroscope (48 h incubation).

DISCUSSION

The samples of leaves and stems of *O. andina* yielded the dichloromethane, methanolic, ethanolic and aqueous extracts as well

as the hydrodistillation residual water used in the experiments. Among all the extracts evaluated, the CH₂Cl₂ extract at 1:100 was the only one able to diminish the growth of *R. solani*, inhibiting colony area at 59%. With CH₂Cl₂ as diluent, the CH₂Cl₂ extract and fractions 2f and 5f showed antifungal activity; while when acetone was used as the solvent, the CH₂Cl₂ extract showed the highest activity, followed by fractions 2f and 3f. Even if fraction 2f was active in both diluents, it was more suppressive when diluted in CH₂Cl₂. It is known that the effectiveness of plant extracts depends on the presence of active components, which can be obtained with different solvents and extraction techniques. The activity of fraction 5f was markedly higher when diluted in CH₂Cl₂, as it had been the extraction solvent, than when diluted in acetone.

This behavior would indicate that a complete dilution of the CH₂Cl₂ extract is hindered by acetone, a solvent with higher polarity than CH₂Cl₂. The differences between assays 2 and 3 in the activity observed for the fractions may be related to their better dilution in CH₂Cl₂.



Different letters indicate differences between treatments. Kruskal-Wallis test, α : 0.05.
 Letras diferentes indican diferencias entre tratamientos. Prueba Kruskal-Wallis, α : 0,05.

Figure 6. Beetroot seeds infected by *R. solani* in dichloromethanic (CH₂Cl₂) extract-treatment.

Figura 6. Semillas de remolacha infectadas con *R. solani* en tratamientos con extracto diclorometánico (CH₂Cl₂).

Table 2. Beetroot germination and soil infestation after seed ball and soil treatments with the dichloromethanic (CH₂Cl₂) extract from *Ovidia andina*.

Tabla 2. Germinación y grado de infestación de suelo luego de aplicar extracto diclorometánico (CH₂Cl₂) de *Ovidia andina* a glomérulos de remolacha y suelo.

Treatment	Germination of beetroot seeds ¹ (%)		Population of <i>Rhizoctonia solani</i> in soil ² (No propagules g ⁻¹)
	day 14	day 21	
H ₂ O	87a	87a	0.020a
CH ₂ Cl ₂	86a	86a	0.010a
CH ₂ Cl ₂ extract 1:100	51b	84a	0.002b

Different letters within a column indicate differences between treatments (¹Satterwaite test, ²DGC test, α : 0.05).
 Letras diferentes dentro de cada columna indican diferencias entre tratamientos (¹prueba Satterwaite, ²prueba DGC, α : 0,05).

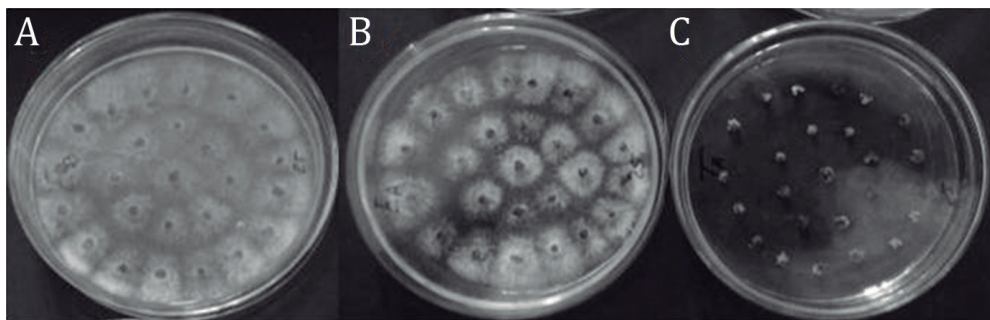


Figure 7. Colonies of *Rhizoctonia solani* on potato dextrose agar, developed from beetroot seed balls incubated in soil treated with A. water. B. dichloromethane (CH_2Cl_2). C. CH_2Cl_2 extract.

Figura 7. Colonias de *Rhizoctonia solani* en agar papa glucosa, desarrolladas a partir de glomérulos de remolacha incubados en suelo tratado con A. agua. B. diclorometano (CH_2Cl_2). C. extracto CH_2Cl_2 .

According to the results, fractions 2f and 5f would contain the most active compounds present in the CH_2Cl_2 extract. The higher or similar activity of the extract compared with its fractions could be attributed to compounds' synergy, which may potentiate each other's action. A similar effect had been observed (4) for a combination of a coumarin with other compounds from fruits of *Melia azedarach*, that strengthened individual antifungal effects against *Fusarium verticillioides*.

Phenolics are a large class of secondary metabolites produced by plants, that include defense-related flavonoids and coumarins. TLC is useful for the analysis of phenolics, and silica gel is the most frequently used adsorbent (33). The chromatographic analysis of the fractions obtained from the CH_2Cl_2 extract showed the presence of flavonoids and coumarins, which could be related to the antifungal behavior observed, in coincidence with Montagner *et al.* (2008). Free flavonoid aglicones exuded by plant tissues may be washed from the surface with nonpolar

solvents, while more polar glycosidic conjugates dissolve in polar solvents (28). This differential behavior may indicate the type of compound that is active in the studied CH_2Cl_2 extract from *O. andina*.

Coumarins also constitute an important group of natural compounds (18) derived from the lactone of the o-hidroxi cinnamic acid, and may be free or glycosylated in the plants (5). The successive use of solvents with increasing polarity has been effective for their extraction and isolation (35). In this work the plant material was macerated with CH_2Cl_2 in order to obtain the CH_2Cl_2 extract, which would be expected to contain low polar and moderately polar compounds present in this species. The vegetable drug was then dried and extracted with methanol. Consequently, the MeOH extract contains the polar compounds present. In addition to a CH_2Cl_2 extract, a hydroalcoholic extract and a decoction were evaluated, given that the solvents used to obtain them are the most frequently used because of their low toxicity on human beings (6).

The potential of wild plants as sources of phenolics to be utilized as natural anti-infective agents in the agricultural industry remains largely untapped (24). They are barriers to fungal penetration in plant tissues (11), and have provided control of *R. solani* (2, 12, 18, 34). The current work reports morphological alterations and reduced colony growth of *R. solani* in the presence of a dichloromethane extract of *O. andina* for the first time. Coincidentally, Plodpai *et al.* (2013) observed globular structures in cells of *R. solani* caused by a dichloromethane extract from *Desmos chinensis*. The flavonoids and coumarins we have detected in the extract might be some of the active antifungal compounds. The activity of the fractions obtained from the CH₂Cl₂ extract differed from each other. This behavior may be attributed to the purity of each fraction or to the synergism among its compounds (14).

Seeds of vegetables, ornamentals or field crops contaminated or infected with *R. solani* may decay or may produce infected seedlings from which the fungus spreads into the soil or to adjacent seedlings. It was proved in this work that *R. solani* contamination of beetroot seed balls may be reduced by immersion in the CH₂Cl₂ extract diluted in CH₂Cl₂ for 120 seconds. The extract delayed germination when evaluated at day 14, but was not significantly different from the control by 21 days after immersion in CH₂Cl₂ extract. The germination of *B. vulgaris* is very sensitive to chemical conditions in the seed bed (15). Talukder *et al.* (29) observed a delay in germination after exposing seeds of *B. vulgaris* to plant extracts. This behavior could be attributed to allelopathy in the extract compounds (20). In this work, the phenomenon was temporary.

Like many other pathogens, *R. solani* has the ability to grow on or through the soil. It was demonstrated that the CH₂Cl₂ extract from *O. andina* inhibits fungal growth on agar media and reduces the inoculum in the soil. *R. solani* is not transmitted by beetroot seeds but is likely to be transported by seed balls after contact with infested soil or plants. The baiting method used throughout this work proved to be sensitive for detecting and quantifying *R. solani* in extract treatments on seed balls and soil. The use of beetroot seed balls as well as wooden toothpicks (22) as baits, are simple and inexpensive techniques that can be used for the detection of *Rhizoctonia* spp. in different studies.

These results constitute the basis to continue the phytochemical research, with the aims of obtaining pure active compounds and fulfilling studies on mechanisms of action on the pathogen. Additionally, further studies could be done to test the effect of the extract on other isolates of the pathogen, which may include other anastomosis groups as well as different soilborne species.

CONCLUSIONS

The native species *O. andina* has arisen as a promising source of anti-fungal compounds. This work demonstrates that the dichloromethane extract of *Ovidia andina* reduces colonization of the beetroot fruits after exposing to high levels of *Rhizoctonia solani* in the soil and controls soil infestation; and that is harmless to the seeds and seedlings.

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Corn silage production in the northern oasis of Mendoza, Argentina

Producción de maíces sileros en el oasis norte de Mendoza, Argentina

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Nota científica

ABSTRACT

Meat production in irrigated areas in Mendoza has increased over the last years. Corn silage emerges as an important forage alternative. This paper evaluates three corn hybrids for silage (ACA 417 RR2, ACA 485 MGRR2, ACA 498 MGRR2) cultivated in the northern oasis of Mendoza during the 2015-2016 and 2016-2017 crop cycles. The study was carried out at the agricultural experiment station of the Facultad de Ciencias Agrarias of Universidad Nacional de Cuyo in Luján de Cuyo, Mendoza (33°00'38" S y 68°52'28" W). No differences were detected in green matter (GM) or dry matter (DM) yields among the hybrids during the crop cycles. In the 2015-2016 crop cycle average dry matter yields and green matter yields were 73,760 kg ha⁻¹ and 23,493 kg ha⁻¹, respectively, whereas during the 2016-2017 crop cycle green matter yields and dry matter yields were 58,390 kg ha⁻¹ and 21,798 kg ha⁻¹, respectively. Genotypes were characterized according to plant height, number of ears of corn, and the ear dry weight/whole plant dry weight ratio. The quality of the three hybrids was also determined. Results obtained suggest that corn silage is a valid forage resource for intensive cattle farming in Mendoza.

Keywords

forage • corn silage • nutritional quality • *Zea mays*

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RESUMEN

La producción de carne bajo riego en Mendoza creció en los últimos años. El silaje de maíz aparece como una alternativa forrajera importante. En este trabajo se evaluaron tres híbridos de maíz (ACA 417 RR2, ACA 485 MGRR2, ACA 498 MGRR2) para silaje cultivados en el oasis norte de Mendoza, durante las campañas 2015-2016 y 2016-2017. El ensayo se realizó en el campo experimental de la Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Luján de Cuyo, Mendoza; 33°00'38" S y 68°52'28" O. No se obtuvieron diferencias de rendimiento de materia verde ni materia seca entre los híbridos en las campañas evaluadas. En la campaña 2015-2016 el rendimiento de materia verde promedio fue de 73.760 kg/ha y el de materia seca de 23.493 kg/ha; mientras que en la campaña 2016-2017 fueron de 58.390 kg/ha de materia verde y de 21.798 kg/ha de materia seca. Se caracterizaron los genotipos evaluados de acuerdo con: altura de plantas, número de mazorcas por planta, relación peso seco espiga/peso seco planta entera, y se determinó la calidad de los tres híbridos. Los resultados obtenidos confirman que el silaje de maíz es un recurso forrajero válido para intensificar la ganadería en la provincia.

Palabras clave

forraje • silaje de maíz • calidad nutricional • *Zea mays*

INTRODUCTION

One of the greatest problems cattle farms are confronted with is lack of feed at certain stages of the production cycle due to the seasonal nature of pasture. In order to ensure forage of high quality and yield to meet beef cattle requirements, preserved forages are used (2).

Corn silage is mainly used as the basic diet of feedlot dairy cattle and steers to supplement forage shortages in winter or the nutritional imbalances of pastures (1). The importance of corn silage as a forage resource lies in the possibility of providing a high quality, high-energy daily food ration year-round which is easy to produce and store, and is very well accepted by the animals (1).

One of the factors that affect corn silage production is the hybrid used. Each hybrid has a production potential that depends on the soil and climate where it is grown. Thus, it is highly useful to know

the performance of corn hybrids under the environmental conditions in the region. Selection of the hybrid will depend on the production potential of the environment and on crop management (12).

The performance and nutritional quality of corn silage has been evaluated in different livestock farms (3, 5, 13, 14, 15). Most of these evaluations were undertaken under rainfed conditions at INTA's experiment stations in the provinces of Buenos Aires, Santa Fe, Entre Ríos and Córdoba. However, there is record of irrigated corn in the province of Neuquén (4).

In Mendoza a few intensive livestock farming undertakings have adopted corn silage in their feeding plans (8, 17) but no experimental results have been published. In 2017 some 3,500 ha were planted with corn silage, representing a significant increase when compared to previous crop cycles (7).

Historically, livestock farming in Mendoza has focused on cattle breeding on natural pastures with an annual production of some 150,000 calves who, after weaning, are sent to provinces in the humid pampas for raising and fattening (10).

However, the province of Mendoza has a number of advantages for raising and fattening on irrigated pastures because its agro-climatic conditions are suitable for growing alfalfa, corn and other forages. Recent studies show that both raising and fattening in feedlots with corn silage as the basic forage would be feasible production alternatives from the technical and economic points of view (9, 10).

Objectives

The objective of this paper is to generate regional information on corn silage production under the farming conditions of Mendoza's northern oasis. Specific objectives are to:

- Compare the production of green matter (GM) and dry matter (DM) per hectare of three corn genotypes.

- Characterize the genotypes assessed according to plant height, number of ears of corn per plant, and the ear dry weight/whole-plant dry weight (DW) ratio.

- Determine the quality of the three genotypes.

MATERIALS AND METHODS

The study was carried out at the agricultural experiment station of the Facultad de Ciencias Agrarias of Universidad Nacional de Cuyo in Luján de Cuyo, Mendoza (33°00'38" S y 68°52'28" W).

The alluvial soil has a clay-loam texture. Mean annual temperature is 15.7°C, while mean annual rainfall is 248.4 mm (Estación Meteorológica Chacras de Coria, 1959-2013).

During the 2015-2016 and 2016-2017 crop cycles three corn hybrids were grown: ACA 417 RR2, ACA 485 MGRR2, and ACA 498 MGRR2. Crop management is shown in the table 1.

Table 1. Cultural management of experimental corn plots, 2015-2016 and 2016-2017 crop cycles.

Tabla 1. Manejo cultural de las parcelas experimentales de maíces, campañas 2015-2016 y 2016-2017.

	2015-2016 crop cycle	2016-2017 crop cycle
Soil preparation	Cross harrowing & furrowing	Cross harrowing & furrowing
Sowing date	11/17/2015	10/25/2016
Fertilization: diammonium phosphate (at sowing)	150 kg	150 kg
Weed control: glyphosate at 2 %	2 applications	1 application
Lepidoptera control: chlorpyrifos 0.6 l ha ⁻¹	2 applications	Not needed
Water depth applied	Thirteen 30 mm irrigations: 390 mm Rainfall: 308 mm Total: 698 mm	Twelve 30 mm irrigations: 360 mm Rainfall: 226.6 mm Total: 586.6 mm
Harvest date	3/8/2016	3/7/2017

The experimental plots had four 10 m-long furrows 0.60 m apart; 5 seeds per linear meter were sown. The experimental design consisted of randomly selected plots with three replications. Plant stand was determined at harvest time, and the plants were harvested when the grain was at the milk-wax stage of ripeness. Only the two central rows in each plot were harvested; the green weight (GW) was determined; and 3 plants from each plot were taken to determine DW and yield components. Five plants per experimental plot were chopped up to obtain the sample on which forage quality was analyzed at the laboratory. Quality variables included crude protein (CP) and acid detergent fiber (ADF). The values obtained were used to calculate digestibility (Dig) with the following formula:

$$\% \text{IVDDM} = 88.9 - (\% \text{ADF} \times 0.779)$$

Energy concentration (EC) of the chopped-up forage was calculated with the following formula:

$$ME = 3.61 \times \text{IVDDM}$$

The results obtained from the variables were analyzed using the analysis of variance and the comparison of means (Tukey test).

RESULTS AND DISCUSSION

The GM and DM yields of the hybrids evaluated in both crop cycles are shown in tables 2 and 3 (page 373). No differences in yields among genotypes were detected in both crop cycles. In the 2015-2016 crop cycle average GM and DM yields were 73,760 kg ha⁻¹ and 23,493 kg ha⁻¹, respectively, whereas during the 2016-2017 crop cycle average GM and DM yields were 58,390 kg ha⁻¹ and 21,798 kg ha⁻¹, respectively.

Experiences in other parts of the country, though under rainfed conditions,

show lower yields per hectare: 16,025 kg of DM on average for 18 corn hybrids tested at the AER INTA Totoras, province of Santa Fe (13); 15,753 kg per ha⁻¹ on average in tests carried out on 13 corn hybrids in Río Cuarto, province of Córdoba (14); and 12,086 kg per ha⁻¹ of DM in Chascomús, province of Buenos Aires (3). On the other hand, field studies in irrigated areas in the Precordillera (Andean foothills) of Neuquén had corn silage yields of about 12,400 kg ha⁻¹ of MS (4).

As regards dry matter percentage at harvest, differences among hybrids were detected in the 2015-2016 crop cycle: the lower percentage was for ACA 417, the highest was for ACA 498 and ACA 485 was somewhere in the middle of the two. This difference could not be detected visually at harvest (grain 2/3 milk line). In the 2016-2017 crop cycle, no differences were found and the average DM percentage at harvest was 37.04%.

With respect to plant height, in the first crop cycle there were significant differences among hybrids, as shown in table 4 (page 373).

However, in the 2016-2017 crop cycle no differences in height were detected among hybrids (table 5, page 373). Plant height was significantly lower in the second crop cycle.

In the 2015-2016 crop cycle, hybrid ACA 498 had more ears of corn/plant than the rest (table 4, page 373), and in the 2016-2017 crop cycle there were no differences among hybrids: all genotypes bore only one ear of corn per plant.

In the first year the ratio of DW of ears of corn/DW of the whole plant showed no differences among hybrids, the average being 45.9%. In the second year ACA 485 showed a lower ratio while ACA 498 had the highest; the ratio for ACA 417 was somewhere in the middle of the two (table 5, page 373).

Table 2. Green matter (GM) yield, dry matter (DM) yield, and percentage of dry matter (DM) of corn hybrids included in the trial. 2015-2016 crop cycle, Luján de Cuyo, Mendoza, Argentina.

Tabla 2. Producción de materia verde (GM) y materia seca (DM) y porcentaje de materia seca (DM) de los distintos híbridos de maíz incluidos en la prueba. Campaña 2015-2016, Luján de Cuyo, Mendoza, Argentina.

Hybrid	Plants at harvest (n°)	GM yield (kg ha ⁻¹)	DM yield (kg ha ⁻¹)	DM (%)
ACA 417	68,824±1,380 a	77,534±6,183 a	23,594±2,329 a	30.43±1.8 a
ACA 485	64,823±8,686 a	69,680±3,338 a	21,927±625 a	31.49±0.68 ab
ACA 498	64,961±7,737 a	74,065±12,651 a	24,957±4,111 a	33.72±0.50 b

Table 3. Green matter (GM) yield, dry matter (DM) yield, and percentage of dry matter (DM) of corn hybrids included in the trial. 2016-2017 crop cycle, Luján de Cuyo, Mendoza, Argentina.

Tabla 3. Producción de materia verde (GM) y materia seca (DM) y porcentaje de materia seca (DM) de los distintos híbridos de maíz incluidos en la prueba. Campaña 2016-2017, Luján de Cuyo, Mendoza, Argentina.

Hybrid	Plants at harvest (N°)	GM yield (kg ha ⁻¹)	DM yield (kg ha ⁻¹)	DM (%)
ACA 417	68,669±5,620 a	57,393±5,972 a	20,560±1,947 a	35.90±2.23 a
ACA 485	66,843±767 a	51,575±11,595 a	18,883±4,745 a	36.48±0.90 a
ACA 498	71,712±2,837 a	66,201±15,354 a	25,950±7,761 a	38.75±3.14 a

Table 4. Plant height, number of ears of corn/plant, and DW ears of corn/DW whole plant ratio of the hybrids included in the trial. 2015-2016 crop cycle, Luján de Cuyo, Mendoza, Argentina.

Tabla 4. Altura de planta, número de mazorcas/planta y relación DW mazorcas/D planta entera de los distintos híbridos de maíz incluidos en la prueba. Campaña 2015- 2016, Luján de Cuyo, Mendoza, Argentina.

Hybrid	Plant height (m)	N° of ears of corn/plant	DW ears of corn/DW whole plant (%)
ACA 417	2.83±0.05 b	1.22±0.19 a	46.80±4.97 a
ACA 485	2.64±0.06 a	1.22±0.19 a	44.79±1.28 a
ACA 498	2.73±0.08 ab	1.78±0.19 b	46.11±8.29 a

Table 5. Plant height, number of ears of corn/plant, and DW ears of corn/DW whole plant ratio of the hybrids included in the trial. 2016-2017 crop cycle, Luján de Cuyo, Mendoza, Argentina.

Tabla 5. Altura de planta, número de mazorcas/planta y relación DW mazorcas/DW planta entera de los distintos híbridos de maíz participantes de la prueba. Campaña 2016-2017, Luján de Cuyo, Mendoza, Argentina.

Hybrid	Plant height (m)	N° of ears of corn/plant	DW ears of corn/DW whole plant (%)
ACA 417	2.45±0.04 a	1.00±0.0 a	30.41±4.70 ab
ACA 485	2.31±0.14 a	1.00±0.0 a	27.83±5.60 a
ACA 498	2.37±0.18 a	1.00±0.0 a	39.65±1.32 b

Table 6. Hybrid quality: gross protein, acid detergent fiber, digestibility and energy concentration. 2015-2016 crop cycle, Luján de Cuyo, Mendoza, Argentina.**Tabla 6.** Calidad de los híbridos (proteína bruta, fibra detergente ácida, digestibilidad y concentración energética), campaña 2015-2016, Luján de Cuyo, Mendoza, Argentina.

Hybrid	Gross protein (%)	Acid detergent fiber -ADF (%)	Digestibility (%)	Energy concentration EC (Mcal kg MS ⁻¹)
ACA 417	7.29±0.83 a	29.38±2.57 a	66.01±2 a	2.38±0.07 a
ACA 485	7.3±0.42 a	29.48±1.7 a	65.94±1.33 a	2.38±0.05 a
ACA 498	6.87±0.38 a	32±2.49 a	63.97±1.94 a	2.31±0.07 a

Table 7. Hybrid quality: gross protein, acid detergent fiber, digestibility and energy concentration. 2016-2017 crop cycle, Luján de Cuyo, Mendoza, Argentina.**Tabla 7.** Calidad de los híbridos (proteína bruta, fibra detergente ácida, digestibilidad y concentración energética), campaña 2016-2017, Luján de Cuyo, Mendoza, Argentina.

Hybrid	Gross protein (%)	Acid detergent fiber - ADF (%)	Digestibility (%)	Energy concentration EC (Mcal kg MS ⁻¹)
ACA 417	7.09±0.33 a	28.44±2.55 a	66.74±1.99 a	2.41± 0.07 a
ACA 485	7.01±0.12 a	28.25±1.98 a	66.89±1.54 a	2.41±0.06 a
ACA 498	6.93±0.40 a	30.44±1.78 a	65.18±1.39 a	2.35±0.05 a

Differences in hybrid characteristics from one year to another may be due to changes in environmental conditions. The amount of water received in 2015-2016 was 111.4 mm larger than in 2016-2017. Besides, the latter cycle was noticeably warmer than the previous one. In December 2015 and January 2016 there were only two days when the maximum temperature was above 35°C whereas in December 2016 and January 2017 there were eleven days with temperatures above 35°C. Environmental conditions during the second crop cycle could be the reason for the lower plant height, for the smaller number of ears of corn per plant, and for the lower ratio of dry weight of ears of corn to whole-plant dry weight because temperatures above 35°C lead to heat stress in corn (11).

Tables 6 and 7 show the results corresponding to the quality of the hybrids evaluated during the 2015-2016 and

2016-2017 crop cycles. No differences were observed in the variables evaluated in both crop cycles. Protein values fall within the expected range (6.8±1.2 % CP) according to Santini, 2016. ADF values also fall within the expected range, and the digestibility calculated from these data is similar to that found by other researchers for other silage hybrids (6, 16). Energy concentration values are slightly lower than those reported by Montesano, 2013 (14).

CONCLUSIONS

It is feasible to achieve high yields from silage corn under irrigation in Mendoza's northern oasis. Similar behavior was observed in all the hybrids tested in both crop cycles. Results suggest that corn silage is an interesting forage resource for intensive cattle farming in the province of Mendoza.

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Composition, physico-chemical properties and antioxidant capacity of *Renalmia alpinia* (Rottb.) Maas fruit

Composición, propiedades físico-químicas y capacidad antioxidante del fruto *Renalmia alpinia* (Rottb.) Maas

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ABSTRACT

Composition and bioactive properties of x'kijit fruit [*Renalmia alpinia* (Rottb.) Maas] pulp and peel is unknown so far. Therefore, the objective of this study was to evaluate physicochemical, and nutritional characteristics, bioactive compounds and antioxidant activity of peel, pulp of the fruit (x'kijit fruit). The fruit pulp was higher in soluble solids (10.53°Brix) and pH (6.2), while the peel had lower pH (3.9) and soluble solids (2.3°Brix). The protein and fat contents were higher in pulp with values 4.2 and 8.6%, respectively. Vitamin C, flavonoids, phenolic compounds, and carotenoids were higher in pulp (28.3 mg ascorbic acid.100 g⁻¹ FW, 567.8 mg catechin.100 g⁻¹ FW, 102.4 mg GAE.100 g⁻¹ FW, and 332 µg of carotenoid.100 g⁻¹ FW, respectively) than peel. However, the peel was higher in anthocyanins (181.4 mg of cyanidin.100 g⁻¹ FW), yielding an antioxidant activity of 23.3mMFe²⁺.100 g⁻¹. The x'kijit fruit demonstrated to have an important nutritional composition and a high antioxidant activity.

Keywords

x'kijit fruit • nutritional characteristics • antioxidant activity

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RESUMEN

Hasta el momento se desconoce la composición y propiedades bioactivas de la pulpa y piel del fruto x'kijit [*Renealmia alpinia* (Rottb.) Maas]. Por lo anterior, el objetivo de este estudio fue evaluar las características fisicoquímicas, nutrimentales, compuestos bioactivos y la actividad antioxidante en la pulpa y piel del x'kijit. La pulpa del fruto presentó mayores valores de sólidos solubles (10,53°Brix) y pH (6,2), mientras que en la piel fueron más bajos pH (3,9) y sólidos solubles (2,3°Brix). Los contenidos de proteína y grasa fueron más elevados en pulpa, 4,2 y 8,6 %, respectivamente. La vitamina C, flavonoides, compuestos fenólicos y carotenoides fueron más elevados en la pulpa que en la piel (28,3 mg de ácido ascórbico 100 g⁻¹ BH (Base Húmeda); 567,8 mg catequina 100 g⁻¹ BH; 102,4 mg GAE.100⁻¹ BH y 332 µg 100 g⁻¹, respectivamente). Sin embargo, en la piel las antocianinas fueron mayores (181,4 mg de cianidina 100 g⁻¹ BH), proveyendo una actividad antioxidante de 23,3 mM Fe²⁺ 100 g⁻¹. La fruta X'kijit demostró poseer una importante composición nutrimental y una alta actividad antioxidante.

Palabras clave

x'kijit • características nutrimentales • capacidad antioxidante

INTRODUCTION

In Mexico, there are more than 23,000 plant species; which are used by rural communities for food, medicine, utensils, alcoholic and non-alcoholic drinks, paper, fiber, and fuel (11). The edaphoclimatic characteristics of the Sierra Norte region of the state of Puebla in Mexico, led to the presence of numerous exotic and medicinal plant species (23). Among the exotic fruits found in this region is *Renealmia alpinia* (Rottb.) Maas, (x'kijit in the Totonaco language), which is one of only four species of the *Renealmia* genera.

The x'kijit fruit typically grows from lowland evergreen jungles up to 1,500 m, and it is an herbaceous plant of 2-6 m high, within florescence of 12-50 cm in length.

The fruit is an ovoid capsule, 3-4 cm long and 1.5-2 cm in diameter. In the immature stage, the fruit peel is red, but turns black when ripe. The pulp has a yellow color with a considerable amount of black seeds (20).

In rural communities of Veracruz, Chiapas, Oaxaca and Puebla, the x'kijit fruit is used for medicine against nausea and vomiting, to prepare ink, and seed oil for frying (22). But in the literature reviewed there are few studies about the evaluations of this fruit for human consumption and other uses. Therefore, the aim of this study was to determine the content of bioactive compounds and antioxidant activity of the pulp and peel of *Renealmia alpinia* (Rottb.) Maas, in order to contribute to the study of composition and applications in industry.

MATERIALS AND METHODS

Fruits and materials

Ripe x'kijit [*Renealmia alpinia* (Rottb. Maas)] fruits were obtained from Cuetzalan del Progreso locality in the Sierra Norte of Puebla, Mexico, in

two different years (November 2015 and 2016). Fruits were selected with no apparent physical and decay damage, washed carefully with distilled water and stored at 8°C in polystyrene bags until the analysis (1). Sodium 2,6-dichloroindophenolate (DCIP), metaphosphoric acid, ascorbic acid, Folin-Ciocalteu reagent, Gallic acid, catechin, 2,4,6-Tripyridyl-s-Triazine (TPTZ), ferric chloride (FeCl₃), Sodium nitrite (NaNO₂), aluminum chloride (AlCl₃), Sodium hydroxide (NaOH), Sodium carbonate (Na₂CO₃), and calcium carbonate (CaCO₃), were purchased from Sigma–Aldrich (MO, USA). Ethanol, methanol, glacial acetic acid, hydrochloric acid, acetone, and sodium acetate were purchased from J. T. Baker (PA, USA).

Physicochemical analyses

The pulp, peel, and seeds were manually separated. The weights of each part of the fruits were determined with an Ohaus, Explorer Pro analytical balance (NY, USA).

The pH, total soluble solids, and titratable acidity of the fruits peel and pulp were evaluated according to 981.12, 932.12, and 942.15 AOAC methods (2), respectively.

The L^* , a^* and b^* color parameters were determined in the pulp (puree) and peel of the fruits in reflectance mode with a Colorflex M6405 colorimeter (Virginia, USA). Hue angle ($\tan^{-1}(b^*/a^*)$) and Chroma ($((a^{*2} + b^{*2})^{1/2})$) values were calculated. Moisture, protein, fat, and ash contents were determined according to 925.25, 12.1.07, 31.04.02, and 31.012 of the AOAC methods, respectively (2), and carbohydrates were calculated by difference. All measurements were performed in triplicate.

Bioactive compounds and antioxidant activity

Each replicate of pulp or peel (0.5 g) was mixed with 25 mL of absolute ethanol or metaphosphoric acid (for vitamin C determination). The mixture was stirred for 30 min at medium speed and filtered to obtain a clear extract. The extract was used to quantify ascorbic acid, flavonoids, total phenols, and antioxidant activity of the x'kijit pulp and peel.

Ascorbic acid was determined by the reduction of 2,6-dichloroindophenol sodium salt (DCIP) according to the method of Julián-Loeza *et al.* (2011), with some modifications. One milliliter of the mixture was placed in an amber tube and mixed with 1 mL of acetate buffer and 1 mL of 2, 6-dichloroindophenol solution (30 ppm). The mixture was stirred for 1 min and evaluated at 515 nm with a UV-Vis Jenway spectrophotometer model 6405 (Staffordshire, UK). The results were reported as mg of ascorbic acid. 100 g⁻¹.

Carotenoid content was evaluated according to Lichtenthaler and Wellburn (1983). One gram of x'kijit pulp and peel were macerated with 5 mL of acetone solution (80% v/v), 2 g of calcium carbonate and 2 g of sea sand; the mixed was centrifuged at 1.096 g at room temperature for 10 min. The absorbance was evaluated at 470, 645, and 662 nm and acetone was used as blank. The contents were calculated according to the following expressions:

$$C_a = 11.75 A_{662} - 2.35 A_{645}$$

$$C_b = 18.61 A_{645} - 3.96 A_{662}$$

$$C_c = 1.000A_{470} - 2.27C_a - (81.4C_b)/227$$

where:

A = absorbance

C_a = chlorophyll a, C_b is the chlorophyll b

C_c = carotenoid content (mg of carotenoids.100 g⁻¹ of FW).

The flavonoid content was determined using 0.5 mL of extract mixed with 0.5 mL of NaNO_2 (1.5%), 1 mL of AlCl_3 (3%), and 1 mL of NaOH (1 N). The mixture was stirred for 5 min and determined at 490 nm.

The result was reported as mg of catechin/100 g⁻¹ of fresh weight (14). Total phenols were analyzed according to the method by Gao *et al.* (2004) with some modifications. One milliliter of the extract was placed in an amber glass tube and mixed with 1 mL of Folin-Ciocalteu reagent (0.1 N). After 3 min, 1 mL of Na_2CO_3 (0.05%) was aggregated.

The mixture was left to stand for 30 min in the dark at room temperature and the absorbance was determined with a UV-Vis spectrophotometer at 765 nm and reported as mg of Gallic Acid equivalent to GAE.100 g⁻¹ FW.

The monomeric anthocyanin in the peel and pulp was made with pH differential method with some modifications (15). One gram of each sample was mixed with 50 mL of methanol/hydrochloric acid (99:1) (v/v). The mixture was stirred for 3 h and filtered. The extracts obtained were diluted with a buffer solution (pH 1.0 and 4.5). The absorbance was evaluated at 520 and 700 nm with a UV-Vis spectrophotometer. The concentration of monomeric anthocyanin was calculated as cyanidin-3-glucoside equivalents (mg. g⁻¹) with the following equation:

$$\text{Anthocyanin (mg. 100g}^{-1}\text{)} = \frac{A \cdot \text{MW} \cdot \text{DF} \cdot 1000 \cdot \text{SF}}{\epsilon \cdot b}$$

where:

A = (Absorbance 520nm - Absorbance 700nm)_{pH 1.0} - (A520nm - A700nm)_{pH 4.5};

MW (molecular weight) = 449.2 mol⁻¹ for cyanidin-3-glucoside (cyd-3-glu)

DF = dilution factor, ϵ (molar extinction coefficient = 26 900 L.mol⁻¹)

SF = (Relationship: Solvent (L)/ Fruit Weithg (100 g⁻¹).

The ferric reducing antioxidant power (FRAP) was quantified according to the method of Ghasenzadeh *et al.* (2012). A portion of the extract (0.75 mL) was mixed with 2.75 mL of FRAP reactive (10 mL of acetate buffer plus, 1 mL of TPTZ (30 mM) and 1 mL of FeCl_3 (60 mM), and incubated at room temperature for 5 min. The mixture was shaken for 1 min and the absorbance was read at 630 nm. The FRAP was quantified as a reduction of iron ions (iron sulfate), and reported as mM Fe^{2+} .100 g⁻¹.

Analysis of data

Results data of physicochemical, nutritive and functional properties was reported as the average of three replicates of each collected season (2015 and 2016). Due to the considerable differences between pulp and peel results were not statistically analyzed among fruit components (peel and pulp).

RESULTS AND DISCUSSION

Physicochemical characteristics

The x'kijit fruits weighed between 10 and 13 g, with contents of 9.3 ± 2.1 , $22.3 \pm 3.8\%$ and $68.5 \pm 5.2\%$ of seed, pulp and peel, respectively. This is typical of fruits in the cactaceae genera (25). The x'kijit fruit is described as an ovoid capsule (3-4 cm of longitude and 1.5-2.0 cm in diameter) with numerous seeds embedded in the pulp (photo, page 381), a similar description was reported by Gómez-Betancur and Benjumea (2014).

The pH and titratable acidity of the peel were 3.9 and $0.33 \pm 0.05\%$ (citric acid), respectively; these values are similar to other fruits, such as bananas and apples.

The total soluble solids content in the pulp (10.5%) and peel (2.3%) were very low compared to other fruits (28) (table 1, page 381).

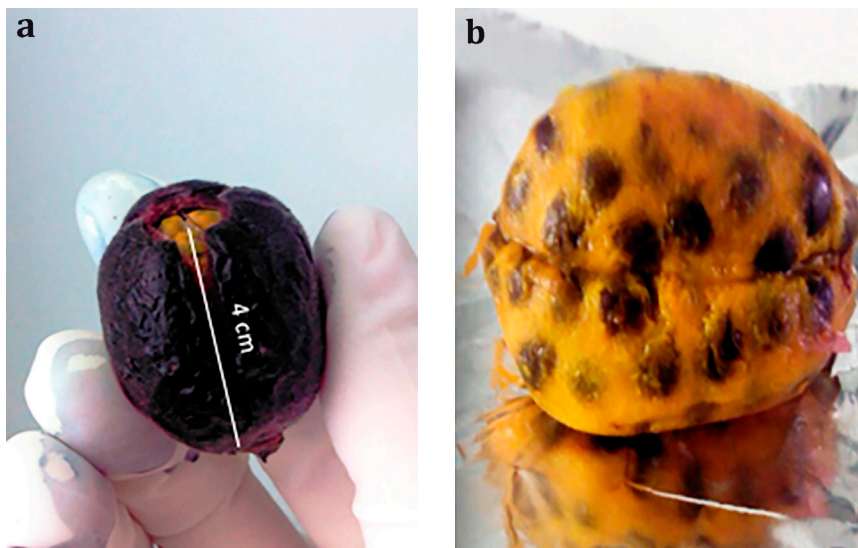


Photo. Whole (a) and pulp and seeds (b) of x'kijit (*Reanealmia Alpinia*) fruit.
Foto. Fruto (a) y pulpa y semillas (b) del x'kijit (*Reanealmia Alpinia*).

Table 1. Physicochemical characteristics of pulp and peel of x'kijit [*Renealmia alpinia* (Rottb.) Maas] fruit^a.

Tabla 1. Características fisicoquímicas de la pulpa y piel del fruto x'kijit [*Renealmia alpinia* (Rottb.) Maas]^a.

Characteristics	Pulp	Peel
pH	6.2 ±0.0	3.9 ±0.0
Total soluble solids (%)	10.53±0.2	2.3 ±0.1
Titrate acidity (%)	0.33 ±0.05	0.37±0.2
<i>L</i> *	49.1 ±0.0	8.2 ±0.2
<i>a</i> *	19.8 ±0.0	23.0 ±0.4
<i>b</i> *	72.7 ±0.3	7.7 ±0.4
Hue	81.9 ±5.5	20.2 ±2.8
Chroma	75.5 ±6.2	22.1 ±3.1

^a Average (n = 6) ± standard deviation. / ^a Promedio (n=6) ± desviación estándar.

The L^* , a^* , and b^* color parameters in pulp were 49.1, 19.8, and 72.7, respectively (table 1, page 381). The Hue angle (82°) indicates an intense yellow color of the pulp and the Chroma value (76) shows high intensity and purity (photo, page 381). The peel presented low L^* , Hue and Chroma values in comparison with the pulp, these results are related to a dull, red color (table 1 and photo, page 381).

Bioactive compounds and antioxidant activity

The x'kijit pulp presented a higher concentration of protein, fat, and carbohydrates than other fruits (table 2). The average protein contents in fruits with high amounts of seeds, comparable to the x'kijit, such as *Annona diversifolia* and *Psidium guajava*, were 0.97% and 2.2%, respectively (14). Fat in x'kijit pulp was high, similar to *Psidium guajava* (27). The carbohydrate content in pulp was higher in contrast to the peel; both values were same to other fruits (28). The moisture

content was higher in the peel than in the pulp; this may be attributed to the elevated amount of protein and fat in the pulp.

Color of the fruits is a parameter related to the content of bioactive compounds (9, 16, 18). The x'kijit yellow pulp and the red peel indicated the presence of bioactive compounds such as flavonoids, carotenoids and anthocyanins (table 2).

The pulp had a medium vitamin C content, consistent with those reported by Contreras *et al.* (2011), and Sánchez Moreno (2006). The ascorbic acid in pulp was about five times higher than in peel (table 2); this content was analogous to other tropical fruits, such as yellow mombin (6). In addition, the contents of ascorbic acid were higher than those reported in tomato, mountain papaya, prickly pear, and tree tomato (3, 8, 18).

Flavonoids are attributed different health benefits, such as arterial dilator, hepatoprotective, antioxidant, choleric, and antifungal characteristics, among others (24).

Table 2. Nutritional and bioactive components of pulp and peel of x'kijit [*Reinealmia alpinia* (Rottb.) Maas] fruit ^a.

Tabla 2. Compuestos nutrimentales y bioactivos de la pulpa y piel del x'kijit [*Reinealmia alpinia* (Rottb.) Maas] fruit ^a.

Proximate composition ^b (%)	Pulp	Peel
Water	76.60± 2.40	94.80±0.60
Protein	4.20± 0.10	0.70±0.00
Fat	8.60± 0.10	2.50±0.40
Carbohydrates	10.30± 2.20	1.90±0.20
Ash	0.40± 0.10	0.10±0.00
Antioxidant compounds		
Vitamin C (mg of ascorbic acid. 100 g ⁻¹)	28.30± 4.00	5.90± 0.40
Flavonoids (mg of catechin. 100 g ⁻¹)	567.80±12.1	55.80± 8.70
Phenolics (mg of GAE. 100 g ⁻¹)	102.40±10.50	59.90± 1.20
Carotenoids (µg 100 g ⁻¹)	332.00±21.70	0.59± 0.24
Anthocyanins (mg of cyanidin.100 g ⁻¹)	ND	181.40±19.40
Antioxidant activity (FRAP) (mM Fe ²⁺ . 100 g ⁻¹)	1.9±0.50	23.3 ± 0.10

^a Mean (n = 6) ± standard deviation. ^b Fresh weight (FW). /^aMedia (n=6) ± desviación estándar. ^bPeso Fresco (PF).

Flavonoids of x'kijit pulp were higher than those in several mango varieties (19) while the phenolics compounds were similar to other fruit contents (21).

These antioxidant compounds evaluated in pulp were ten and two times higher than in peel, respectively (table 2, page 382).

The carotenoid in the x'kijit pulp ($332 \mu\text{g } 100 \text{ g}^{-1}$) was higher than in the peel ($0.59 \mu\text{g } 100 \text{ g}^{-1}$) (table 2, page 382); this compound provides pulp a deep yellow pigmentation (4). Anthocyanins were not detected in pulp, but the peel showed a high concentration ($181.4 \text{ mg of cyanidin.}100 \text{ g}^{-1}$) (table 2, page 382), these results are comparable to those reported in other peels (skin) and whole fruits (5, 7, 26). Thus, the x'kijit pulp and peel have several uses in food, cosmetics, pharmaceutical and medicine, as those practiced by local people in rural communities (24).

Antioxidant properties

The FRAP value on the peel was superior to the pulp ($23.3 \text{ mM Fe}^{2+} \cdot 100 \text{ g}^{-1}$) (table 2, page 382), probably it is due to the peel's

high anthocyanins content. Moreover, this antioxidant property is considered in the intermediate and high levels compared to other fruits (30, 31).

CONCLUSION

It is estimated that this study is the first to characterize the x'kijit fruit, that includes physicochemical properties and nutritive and functional components. The pulp of x'kijit is rich in protein and fat. Besides it has strong antioxidant potential due to their high content of flavonoids, carotenoids in pulp and anthocyanins, phenolic compounds in peel. X'kijit fruits are a new nutritional alternative, a natural source of pigments and bioactive compounds with antioxidant properties.

These properties could help elucidate commercial uses and products of the fruit components in the pharmaceutical and food industries.

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Análisis bibliométrico de la producción científica de la Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Argentina

Bibliometric analysis of the scientific production of the Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo, Argentina

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RESUMEN

Se analizó la producción científica de los investigadores de la Facultad de Ciencias Agrarias (FCA) de la Universidad Nacional de Cuyo, Argentina, con el fin de contar con datos bibliométricos en el área de Ciencia y Técnica. Del análisis de 100 proyectos de investigación financiados por la Secretaría de Ciencia y Técnica de la Universidad Nacional de Cuyo se desprende que el 66% de los proyectos presentan producción de artículos científicos, de los artículos publicados el 46% se hizo en revistas con factor de impacto (FI). Las subdisciplinas en las cuales hubo más producción fueron las Ciencias Biológicas (Botánica, Ecología, Fisiología, Genética, Bioquímica, Biología Molecular) (20%) y Producción Vegetal (17%). El análisis de la producción de artículos científicos en la base de datos Scopus muestra una baja producción científica entre los años 1996 y 2000 y un incremento importante y sostenido de la producción desde el año 2001 en adelante. Este incremento se puede explicar en parte por la radicación, a partir del año 2000, de investigadores en la FCA que estaban trabajando en otras Universidades Nacionales o en el extranjero y por la producción de investigadores que forman parte de institutos del CONICET.

Palabras claves

Producción científica • Bibliometría • Universidad Nacional de Cuyo • Agronomía

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ABSTRACT

The scientific production of the researchers of the Facultad de Ciencias Agrarias de la Universidad Nacional de Cuyo, Argentina, was analyzed. Only 66% of the 100 scientific projects evaluated had published scientific articles, from this 46% were published in journals with impact factor (IF). Biological science which includes Botany, Plant Physiology, Ecology, Plant Genetics, Biochemistry, Molecular Biology and Plant Production were the subjects with more production, 20 and 17%, respectively. The analysis of the scientific production using the SCOPUS database showed a low scientific production during 1996 to 2000 period and a sustained increase of article published from year 2000 until now. The increment observed could be explained, in part, by the incorporation of researchers since year 2000 that were working or studying in other Universities of Argentina or abroad and by the production of researchers from institutes from CONICET.

Keywords

Scientific production • Bibliometric • Universidad Nacional de Cuyo • Agronomy

INTRODUCCIÓN

La evaluación de la producción científica-tecnológica es una herramienta fundamental para diseñar políticas de desarrollo de las Universidades en el mundo. A nivel mundial existe una tendencia a evaluar las instituciones educativas de nivel superior con el fin de mejorar la calidad del sistema educativo y de investigación. Según Hazelkorn (2009) esto se debe en parte a la globalización de la educación superior y a la necesidad creciente de información tanto de estudiantes como de los gobiernos y la sociedad que demandan un mayor compromiso de las Universidades con el medio en el que se desenvuelven. A nivel internacional existen organizaciones que elaboran rankings de instituciones universitarias como por ejemplo el Academic Ranking of World Universities (conocido como Shanghai Index o AWRU), el World University Ranking del Times Higher Education Supplement (THES-Ranking) o QS World University Rankings® elaborado por una consultora privada del Reino Unido. En Argentina, desde el año 1996 funciona la Comisión Nacional de Evaluación y

Acreditación Universitaria (CONEAU), es un organismo público dependiente de la Secretaría de Políticas Universitarias del Ministerio de Educación, encargado de la evaluación de las universidades públicas y privadas y la acreditación de sus respectivas carreras de grado y posgrado.

La producción científica y tecnológica de una institución se puede evaluar a través del análisis de la asignación de recursos, la cantidad de trabajos científicos publicados, el número de patentes o el número de investigadores entre otros parámetros. El análisis de las publicaciones científicas se ha convertido en una herramienta fundamental a la hora de evaluar la calidad de las instituciones Universitarias (5). En la actualidad se cuenta con una serie de herramientas bibliométricas que permiten analizar la producción científica. Uno de los aspectos que se evalúa es la cantidad de trabajos científicos publicados por los investigadores de las instituciones y el número de citas. Para este análisis se utilizan diferentes métodos como por ejemplo el factor de impacto (FI), entre otros (1).

Hasta ahora no se ha realizado un análisis de la producción científica tecnológica de la FCA. Contar con información sobre el tipo y calidad de la producción en Ciencia y Técnica es fundamental para establecer políticas futuras para el desarrollo de la institución. Los objetivos de este trabajo son: a) analizar la producción científica tecnológica de la FCA sobre la base de los informes de los proyectos de investigación financiados por la Secretaría de Ciencia, Técnica y Posgrado de la UNCuyo en el período 2011-2013 y b) evaluar la producción científica de la FCA a través de las bases de datos Scopus (Elsevier).

MATERIALES Y MÉTODOS

Se relevó la producción de trabajos científicos declarados en los informes finales de los proyectos financiados por la UNCuyo a través de la Secretaría de Ciencia, Técnica y Posgrado y se analizaron los siguientes parámetros: a) investigadores por proyecto, b) filiación de los investigadores, c) publicaciones por investigador, d) disciplina en la que se publicó el trabajo, e) tipo de publicación (libro, capítulo de libro, artículo científico).

Las revistas en las cuales se publicaron los artículos científicos se clasificaron en revistas con o sin FI y aquellas que contaban con FI se categorizaron en cuartiles según la importancia de la revista en el área del conocimiento. Este análisis se realizó a través del indicador desarrollado por Scimago Journal Rank (SJR) que representa la visibilidad de las revistas contenidas en la base de datos Scopus desde 1996 (2).

El análisis de la producción histórica de la FCA se realizó en la base de datos Scopus, una de las bases de datos científicas más importante del mundo, que cubre revistas incluidas en la base de datos de Thomson Reuters Web of Science, entre otras.

RESULTADOS Y DISCUSIÓN

Producción en Ciencia y Tecnología de la FCA período 2011-2013

Durante el período 2011-2013 se ejecutaron 100 proyectos de investigación en la FCA financiados por la UNCuyo, el 66% de proyectos presentaron producción de artículos publicados en revistas científicas, artículos de difusión y capítulos de libro. Participaron un total de 494 investigadores que publicaron 162 trabajos, 33,6% de los autores pertenecen a su vez a otras instituciones como el Instituto Nacional de Tecnología Agropecuaria (INTA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) y otras Universidades Nacionales, entre las más importantes (tabla 1).

Tabla 1. Instituciones a la que pertenecen los autores de artículos en colaboración.

Table 1. Author affiliation of coauthored articles.

Instituciones	Número de investigadores	Porcentajes
INTA	46	27,7
CONICET	36	21,6
Universidades Nacionales	25	15,0
Otras Facultades de la UNCuyo	14	8,4
Universidades Extranjeras*	12	7,2
INTI	10	6,0
INV	9	5,4
Empresas	9	5,4
INA	5	3,0
Total	166	100

* Entre las Universidades extranjeras se menciona: Universidad de Chile, Universidad de Bari, Italia, Universidad della Tucsia, Italia.

*Among the international Universities it can mention: Universidad de Chile, Universidad de Bari, Italia, Universidad della Tucsia, Italia.

Del total de artículos, el 46% se publicaron en revistas indizadas según la bases de datos Scimago Institution Ranking en el área de agronomía (6), el resto correspondió a artículos en revistas no indizadas, capítulos de libros y publicaciones de difusión (figura 1). El 60% de los trabajos publicados en revistas indizadas se encuentra entre el primer y segundo cuartil en importancia en el área según la misma base de datos. Del análisis de la producción total de publicaciones por subdisciplina se destacan en primer lugar las relacionadas con Ciencias Biológicas como Botánica, Biología, Fisiología, Genética, Bioquímica y Biología Molecular con un 20% de los trabajos, luego se ubican trabajos relacionados con Producción Vegetal (17%) y finalmente el área de Economía y Sociología Rural (13%).

Los trabajos relacionados con disciplinas como clima, suelo, agua y ambiente suman un 19% de los trabajos, las disciplinas Enológicas y Agroalimentarias cuentan con 10 y 8%, respectivamente (figura 2, pág. 391). Durante este período la UNCuyo invirtió en total \$1.500.000 (\$15.000 por proyecto bianual), lo que resulta en una inversión aproximada de \$20.833 por trabajo publicado en revistas indizadas. Otra fuente importante de financiamiento es la Agencia Nacional de Promoción Científica y Técnica (ANPCyT), según datos de la Fundación de la UNCuyo ocho proyectos financiados por ANPCyT, entre 2013 y 2015, cuyos investigadores responsables pertenecen a la FCA, cuentan con una financiación total de \$3.500.000. De acuerdo con la base de datos SCOPUS en el período 2011-2013 los investigadores de la FCA publicaron 64 trabajos que recibieron 597 citas.

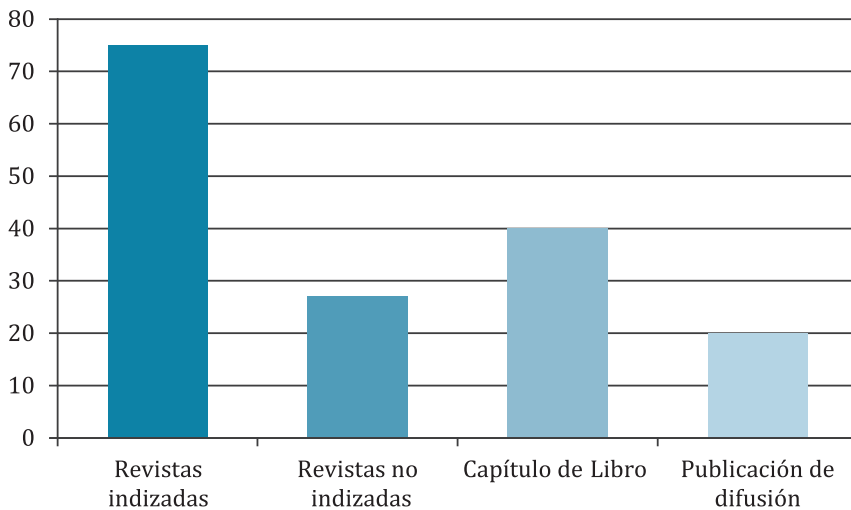


Figura 1. Producción de artículos científicos y de difusión de investigadores de la FCA declarados en los proyectos SECTyP en el período 2011-2013.

Figure 1. Scientific articles and diffusion from researchers of the FCA registered in the SECTyP, 2011-2013.

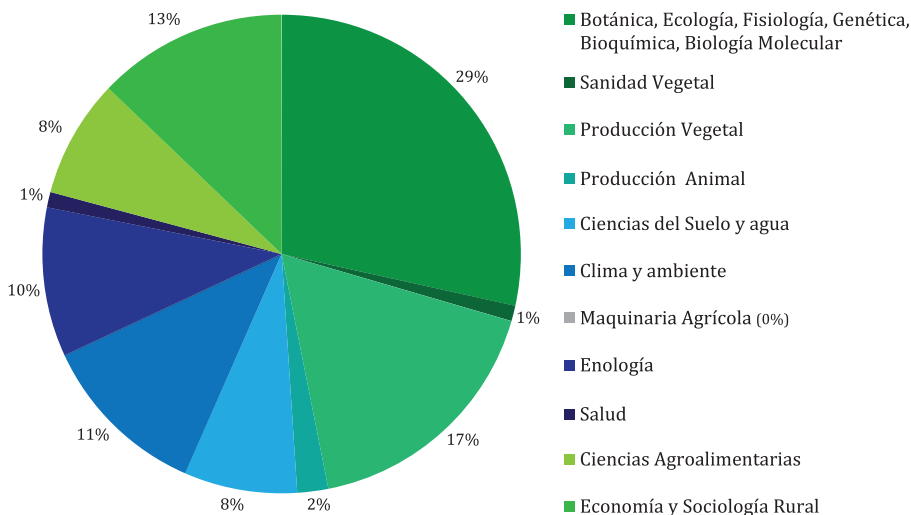


Figura 2. Distribución porcentual por subdisciplina de artículos científicos y de difusión de investigadores de la FCA declarados en los proyectos SECTyP en el período 2011-2013.

Figure 2. Distribution by subject area of the scientific and diffusion articles from researchers of the FCA registered in the SECTyP, 2011-2013.

Análisis de la producción científica de la FCA de acuerdo con la base de datos SCOPUS

La base de datos SCOPUS registra producción científica de la FCA desde 1968 hasta la fecha, con una producción total de 219 publicaciones que recibieron 2658 citas. Si se analizan los resultados presentados en la figura 3 (pág. 392), a partir de 1996, fecha desde la que SCOPUS mantiene información bibliográfica más actualizada, se puede ver que en ese año figuran 3 trabajos llegando a un pico, con altibajos según años considerados, de 27 trabajos en el 2011 y 2014. Se nota una clara tendencia creciente en el número de trabajos publicados desde el 2000 hasta la fecha (figura 3, pág. 392). Esta tendencia se puede explicar por la incorporación a la FCA de investigadores que a su vez pertenecen a otros organismos nacionales de ciencia y técnica, principalmente del Consejo Nacional de

Investigaciones Científicas y Técnicas (CONICET). Según esta base de datos los diez autores más productivos de la institución a su vez pertenecen al CONICET (datos no mostrados).

Un aspecto importante a tener en cuenta en el análisis de la producción en ciencia y técnica son las colaboraciones internacionales. Entre los tres primeros países que se comparte la autoría de los trabajos figuran, España, Estados Unidos y Brasil con 16, 16 y 3 documentos, respectivamente (figura 4, pág. 392). En este sentido, el número de colaboraciones a nivel internacional es baja, solo en el 20,4% de la publicaciones participan investigadores extranjeros. Sin embargo, las colaboraciones con instituciones nacionales representan el 99% de los trabajos publicados y participan investigadores de instituciones como CONICET, INTA y Universidades Nacionales, entre otras.

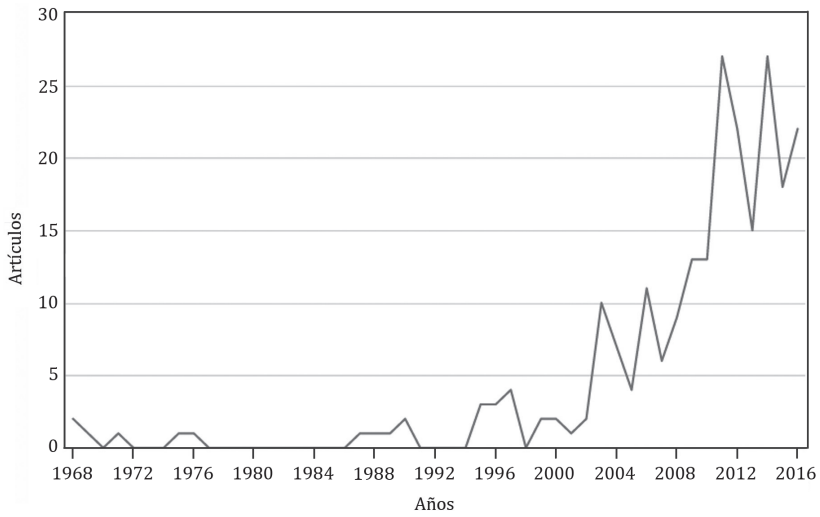


Figura 3. Artículos publicados por investigadores de la FCA desde 1968 a 2016, recopilados por la base de datos Scopus. Los datos corresponden a la visita de la página de Scopus del día 09/08/16.

Figure 3. Scientific articles covered by the scientific database Scopus from 1968 to 2016. The data correspond to the date 08/09/2016.

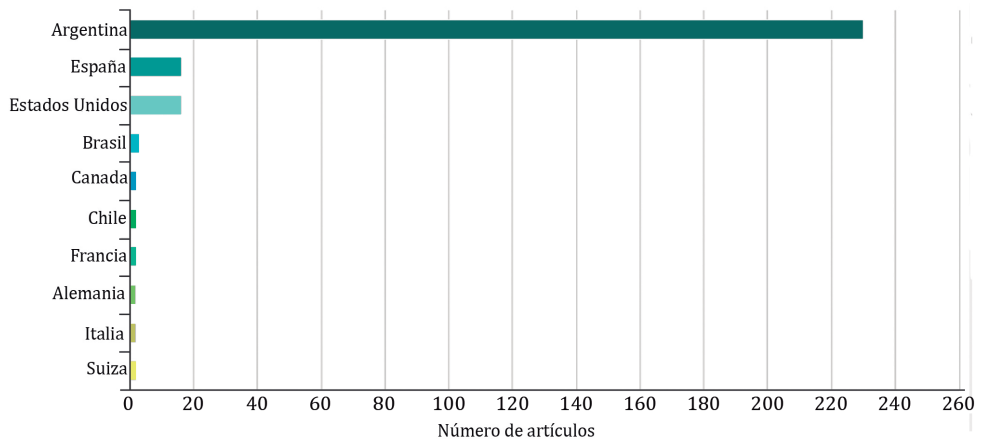


Figura 4. País de origen de los autores de artículos en colaboración.

Figure 4. Country of the authors in coauthorship articles.

La base de datos Scopus tiene registros incompletos de publicaciones antes de 1996, sin embargo es importante destacar el incremento notable en la producción científica en los últimos 16 años comparado con la baja producción que se registra en décadas anteriores.

Varios autores analizan la baja inversión en ciencia en países en vías de desarrollo y se lo atribuyen a diferentes factores. Entre ellos, Vessuri (1995) opina que el principal problema del atraso en ciencia y técnica de los países de América Latina se debe a la cíclica inestabilidad política, social y económica de los países. La UNCuyo y por lo tanto la Facultad de Ciencias Agrarias no escapa a esta situación.

La Universidad a fines de 1949 contó con proyectos cuya finalidad era crear las bases para un sistema científico tecnológico en la UNCuyo. En este sentido, se creó el Departamento de Investigaciones Científicas (DIC) nucleando a prestigiosos docentes e investigadores nacionales y extranjeros en áreas como Física, Matemática, Química y Biología. En particular el instituto de Matemática tuvo reconocimiento internacional convirtiéndose en una de los centros más importantes de investigaciones en matemática (3).

Este impulso que se le dio a la Ciencia y la Tecnología en la región de Cuyo entre 1940 y 1950 se vio abortado por el golpe militar en el año 1955 y obligó a que muchos investigadores emigraran. Esta situación se repitió en las décadas siguientes, siendo el golpe militar de 1976 el que terminó de desarticular la estructura de ciencia y técnica de las Universidades Nacionales, exonerando a gran parte de los docentes investigadores activos en aquellos años. Esto sin duda afectó la producción científica de la FCA en los años siguientes ya que se desarticulaban grupos de trabajo productivos en distintas disciplinas.

CONCLUSIONES

El análisis de la producción científica de la FCA sobre la base de los documentos declarados en los informes de los proyectos de investigación muestra que de los 162 documentos publicados, menos de la mitad se publicaron en revistas indizadas. La producción por investigador es baja (0,35 artículo/investigador), esto muestra que los autores de los artículos científicos publicados son una proporción mínima del total de investigadores que participan en el sistema de ciencia y tecnología de la FCA. Esto se comprueba al analizar la producción científica de la FCA desde 1968 a la fecha de acuerdo con la base de datos SCOPUS, se puede ver que los primeros diez autores con mayor producción de la FCA son a su vez investigadores del CONICET e INTA, no se registran investigadores que pertenezcan a la FCA como único lugar de trabajo.

El análisis de la base de datos SCOPUS también muestra un crecimiento prácticamente exponencial desde el 2001 hasta el 2015 en publicaciones en revistas indizadas. Esto se debe en gran parte a la radicación de investigadores que provenían de otras instituciones del país o del extranjero o que volvían de capacitaciones como doctorados y posdoctorados fuera de la FCA.

Entre el año 2000 y el 2015 se radicaron o reintegraron a la FCA cerca de diez investigadores, que en su mayoría formaron el Instituto de Biología Agrícola Mendoza (IBAM) creado en el 2009 como instituto de doble dependencia con la UNCuyo. Entre los aspectos a mejorar se puede citar la necesidad de aumentar las colaboraciones con investigadores a nivel internacional, que permitan aumentar la calidad de la producción científica y la visibilidad de la institución.

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Agronanotecnología: una nueva herramienta para la agricultura moderna

Agronanotechnology: a new tool for modern agriculture

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RESUMEN

La nanotecnología (NT) estudia los materiales cuyo tamaño corresponde a la escala atómica y molecular (1-100 nm), destacando como los más estudiados a las nanopartículas (NPs) metálicas y las derivadas del carbono. En lo que respecta a la agricultura moderna, los estudios relacionados a este tipo de materiales han ido avanzando y se prevé un importante crecimiento en los reportes de resultados de investigación en el área, debido a la posibilidad de incrementar con su uso, la producción de alimentos. En este sentido, la NT brinda la posibilidad de generar nanofertilizantes y promotores del crecimiento. No obstante, actualmente existen imprecisiones sobre los resultados obtenidos, por una parte, algunas investigaciones muestran que la aplicación de los nanotubos de carbono y las NPs metálicas tienen un efecto benéfico en el crecimiento de las plantas, mientras que otros trabajos reportan inhibición y fitotoxicidad. Actualmente, la demanda de alimentos básicos a nivel mundial aumenta, pero las investigaciones y productos para incrementar el rendimiento de los cultivos son aún insuficientes. Por lo tanto, una opción para el desarrollo de una agricultura sustentable pudiese ser la generación de nanoproductos. Debido a lo antes señalado, este artículo analiza recientes investigaciones relacionadas con la utilización de las NPs en la agricultura y sus usos potenciales como nanofertilizantes y promotores de crecimiento de las plantas.

Palabras clave

agricultura sustentable • nanotecnología • nanopartículas

ABSTRACT

Nanotechnology (NT) is focused to study the materials at the atomic and molecular scale (1-100 nm), the most studied are metallic nanoparticles (NPs) and those derived from carbon. In modern agriculture NPs are investigated because they offer the possibility to control plant pathogens and to increase the yield of plant crops. Nanotechnology provides scientific tools to design and prepare important products such as fertilizers and plant growth promoters. However, scientific literature is plenty of mix results; for example, some papers reports the benefits obtained from the application of carbon nanotubes and metal NPs, promoting plant growth effects, while others papers mention inhibition and phytotoxicity on seeds and plants. NT in agriculture to produce nanofertilizers and pesticides is still being investigated; however, derivative products and applications have been steadily increasing in recent years and are expected to continue growing. Currently, the worldwide demand for food is increasing and research and products to enhance crops yield are still insufficient. Therefore, the industry needs to generate nanoproducts for the development of a sustainable agriculture. Consequently, this review presents recent results about the use of NPs in agriculture, and its potential uses as nanofertilizers and plant growth promoters.

Keywords

sustainable agriculture • nanotechnology • nanoparticles

INTRODUCCIÓN

En un intervalo de tiempo relativamente corto, la nanotecnología ha tenido un impacto significativo en numerosos sectores incluyendo la agroindustria. La NT, considerada como una tecnología emergente, se orienta a la caracterización, elaboración y aplicación de materiales de dimensiones muy pequeñas, en el rango de 1 a 100 nanómetros (nm), es decir de 1 a 100 billonésimas de metro (82), por lo que se ubica dentro de la escala atómica y molecular.

La NT es un extenso campo de la investigación interdisciplinaria, ya que con ella se abre un amplio abanico de oportunidades en diversas áreas como la medicina, la industria farmacéutica, la electrónica y la agricultura sustentable (54).

Actualmente los científicos agrícolas se enfrentan a una amplia gama de desafíos como el estancamiento en el rendimiento de los cultivos, la baja eficiencia en el uso de nutrientes, la disminución de la materia orgánica del suelo, las deficiencias de múltiples nutrientes, el cambio climático, la reducción de la superficie agrícola, la baja disponibilidad de agua para riego y la escasez de mano de obra; además del éxodo de personas desde las áreas agrícolas a las ciudades (11).

A pesar de esas enormes dificultades que enfrenta el sector agrícola, es necesario mantener un crecimiento sostenido del 4% para afrontar los desafíos que representa la demanda de alimentos a nivel global (40). Por lo tanto, para hacer frente a esa diversidad de problemas, es necesario explorar una de las tecnologías de vanguardia como la NT. Esta herramienta científica, permite detectar la presencia de plagas y enfermedades,

contaminación de alimentos así como aplicar la cantidad correcta de nutrientes y pesticidas que promuevan la productividad. Al mismo tiempo, garantizan la seguridad del medio ambiente y una mayor eficiencia en el uso de insumos agrícolas (45). En este contexto, se han desarrollado nuevos nanomateriales basados en el uso de NPs metálicas, poliméricas, inorgánicas, etc., las cuales permiten aumentar la productividad y buscan encontrar aplicaciones para el perfeccionamiento de nanosistemas inteligentes para la captura e inmovilización de nutrientes y su gradual liberación en el suelo para acrecentar la eficiencia de los fertilizantes (30).

Esos sistemas tienen la ventaja de reducir al mínimo la lixiviación, al tiempo que mejoran la absorción de nutrientes por las plantas y coadyuvan a mitigar la eutrofización al reducir la transferencia de nitrógeno a los mantos acuíferos subterráneos (38).

Además, es importante mencionar que los nanomateriales también podrían ser explotados para mejorar la estructura y función de los plaguicidas mediante el aumento de la solubilidad, la resistencia contra la hidrólisis, mejorando su foto descomposición y/o proporcionando una manera más eficaz de liberación controlada hacia los organismos objetivo (42).

Por la importancia y posibles beneficios de la agronanotecnología como nueva y emergente área del conocimiento, este artículo de revisión presenta recientes investigaciones relacionadas con la utilización de las NPs en la agricultura y sus usos potenciales como nanofertilizantes, promotores de crecimiento de las plantas, nanoplaguicidas y nanosensores.

POTENCIAL DE LA NANOTECNOLOGÍA EN LA AGRICULTURA Y LOS ALIMENTOS

El potencial de la NT ha sido documentado en la literatura en diversas áreas tales como: en el cuidado de la salud al utilizarse en la fabricación de textiles antimicrobiales o de nuevos materiales con propiedades superiores a los ya existentes.

Adicionalmente, se ha empleado también, en tecnologías de información, comunicación, así como en el sector energético y en la aeronáutica. Sin embargo, la aplicación de la NT en la agricultura apenas ha comenzado a investigarse y validarse con productores y empresarios.

En el caso de la agricultura, la NT ofrece la posibilidad de mejorar la producción agrícola mediante diversas estrategias

que pueden incrementar la producción de alimentos utilizando menores insumos de energía y reduciendo los costos de producción y los desperdicios de agroquímicos (70).

Las aplicaciones de la NT en la agricultura son muy diversas, destacando principalmente la elaboración de nanopesticidas encapsulados para su liberación controlada (22), la producción de macro y micronutrientes al nivel nano, así como su utilización buscando hacer más eficiente y sustentable el uso y aplicaciones de los agroquímicos (45).

El uso potencial de la NT para diseñar y fabricar nanosensores que permiten detectar la presencia de plagas y enfermedades en los cultivos (18) se presenta en la figura 1.

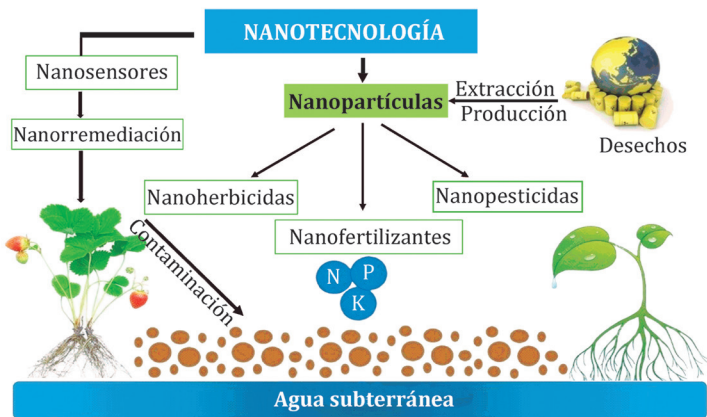


Figura 1. Aplicaciones potenciales de la nanotecnología y de las nanopartículas en la agricultura, incluyendo la fabricación de nanosensores, nanofertilizantes, nanopesticidas y nanoherbicidas.

Figure 1. Potential applications of nanotechnology and nanoparticles in agriculture, including the manufacture of nanosensores, nanofertilizers, nanopesticides and nanoherbicides.

Asimismo, la NT ha permitido el desarrollo de empaques inteligentes de alimentos que pueden revelar el desarrollo bacteriano o fúngico al estar envasados (77).

La figura 1 (pág. 398), también muestra que diversos tipos de NPs metálicas como las de Ag, Fe, Cu, Zn, etc., pueden ser utilizadas con un enfoque dual, ya sea como nanofertilizantes al mejorar la germinación de semillas y al promover el crecimiento de las plantas (26), o para emplearse como nanopesticidas (34). Algunos materiales como arcillas y zeolitas pueden ser usados al nivel nano para mejorar la capacidad de retención de agua en el suelo, y además actúan como materiales de lenta liberación de agua y fertilizantes, incrementando así la eficiencia en el uso de agua y nutrientes por las plantas (69).

En años recientes muchos esfuerzos se han venido realizando en centros de investigación e instituciones de educación superior de todo el mundo, para encontrar novedosas aplicaciones de la NT en la producción sustentable de alimentos y cultivos (10, 28). En este contexto, la nanoencapsulación de agroquímicos y microorganismos benéficos ha adquirido una gran relevancia al ser una manera de controlar plagas y enfermedades con bajo impacto ambiental, lo cual permite reducir la cantidad de agroquímicos que dañen los ecosistemas (22). Es por eso que las inversiones en agricultura y alimentación nanotecnológica van en aumento, debido a que sus beneficios potenciales se enfocan a mejorar la calidad e inocuidad de los insumos agrícolas al ser utilizados en menor volumen y promoviendo

mejoras en la nutrición (61). Mientras que la mayor inversión en NT se realiza en países desarrollados, los avances de investigación permiten vislumbrar posibles aplicaciones en la agricultura, alimentos y en la bioseguridad del agua, lo que podría tener un impacto significativo en la sociedad de países en desarrollo (23).

La observación mencionada se centra en las estrategias modernas utilizadas para el manejo del agua, el uso de agroquímicos convencionales y el potencial de los nanomateriales en la agricultura sustentable, con un enfoque moderno en las fronteras de la agricultura no tradicional (1, 74).

A pesar de que las posibles aplicaciones de la NT son muy vastas, los usos actuales en el sector agrícola y alimentario son relativamente escasos, debido a que la agronanociencia se encuentra en un estado emergente (73). Una visión general de más de 800 productos de consumo basados en NT que actualmente están disponibles a nivel mundial, apunta que solo cerca del 10% de ellos son alimentos, bebidas y productos para el envasado de comestibles (12). Sin embargo, en los últimos años los productos y aplicaciones derivados de la NT en estos sectores han ido en constante aumento y se prevé que siga creciendo rápidamente en el futuro (59).

Todas esas aplicaciones potenciales que han sido generadas por trabajos de investigación, se ven claramente reflejadas en incremento constante de las publicaciones relacionadas con la NT en el sector agrícola y de productos alimenticios (figura 2, pág. 400).

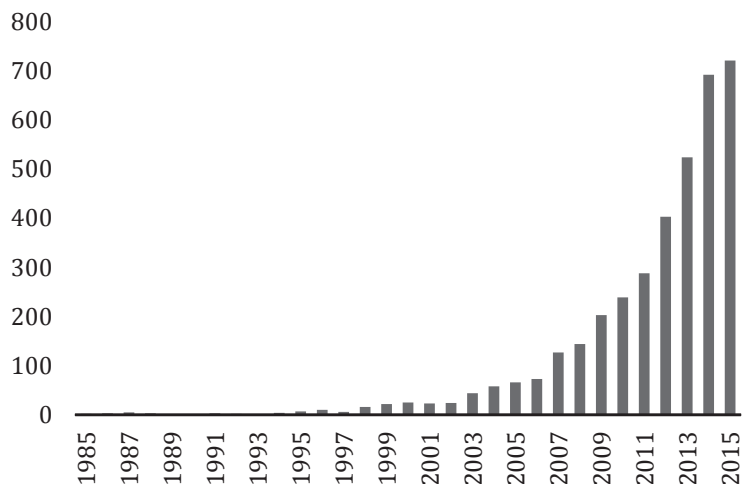


Figura 2. Publicaciones anuales en revistas científicas relacionadas con nanotecnología aplicada a la agricultura y en productos alimenticios.

Figure 2. Annual publications in scientific journals related to nanotechnology applied to agriculture and food products.

PRODUCTOS USADOS EN APLICACIONES AGRONANOTECNOLÓGICAS

Diversos nanomateriales se han utilizado e investigado por su función biológica en células de humanos y plantas, como son los nanotubos de carbono (NTC), fullerenos, liposomas, proteínas y polímeros (figura 3, pág. 401), incluyendo biopolímeros como el quitosán (21).

Los efectos positivos de los NTC en el crecimiento y desarrollo de las plantas han sido documentados por varios grupos de investigación, habiendo reportado que incrementaron el crecimiento de las raíces en plantas de cebolla y pepino (6), así como de zacate rye grass (35).

Además, se ha demostrado que los nanotubos de carbono de pared múltiple (MWCNT), pueden activar el crecimiento de plantas de tomate y afectar a la expresión de genes que son esenciales para la división celular y el desarrollo de la planta (29, 78).

Liu *et al.* (2009), demostraron que los nanotubos de carbono de pared sencilla poseen el tamaño adecuado como para penetrar en las paredes y membranas de las células de tabaco. Esto ha generado interés, porque de manera similar a las aquaporinas pueden ayudar a transportar muy rápidamente agua y nutrientes dentro de las plantas (27).

También son útiles para procesos de desalinización, ya que pueden cargarse internamente con iones de Na^+ y Cl^- para luego eliminar del sistema las sales en exceso (20).

Por su parte los fullerenos al tener una nanoestructura en forma de esfera o balón, pueden utilizarse como contenedores de material biológico o mineral de tal manera que pueda ser transportado hasta su destino en el interior de su estructura, donde luego será utilizado por las plantas (25).

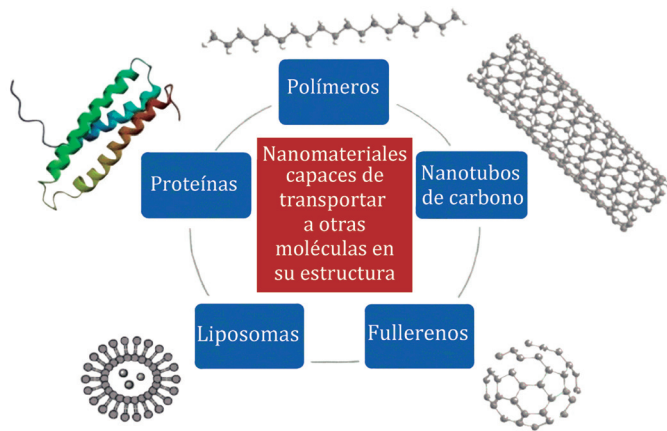


Figura 3. Algunas características de los nanomateriales usados en una gran diversidad de aplicaciones biológicas e industriales.

Figure 3. Some characteristics of nanomaterials used in a wide variety of biological and industrial applications.

Los organelos celulares conocidos como liposomas también han probado ser útiles para almacenar nitratos en su interior y ser transportados dentro de plantas de *Arabidopsis thaliana* (50).

Para el caso de la horticultura moderna, se están utilizando nanomateriales para el tratamiento de algunas enfermedades de plantas, para la detección precoz de los patógenos que las producen (31), para la mejora en la asimilación de nutrientes esenciales (41), e incluso para construir nanobiosensores importantes en determinados procesos biológicos (79).

La NT puede incrementar la eficacia de los pesticidas e insecticidas comerciales, reduciendo la cantidad de aplicación al suelo o follaje a dosis significativamente menores que las requeridas de manera convencional, con la mejora que eso implica para los ecosistemas (81).

La nutrición mineral de las plantas en la agricultura es de primordial importancia y en este ámbito los nanofertilizantes ya están ofreciéndose en el

mercado de Estado Unidos; por ejemplo, la compañía A.M. Leonard, puso a la venta el producto denominado Florikan, el cual se ha llamado NANO 16-5-11.

La empresa que lo produce señala que al aplicar NANO, se obtendrá 10 veces el número de puntos de contacto comparado con el fertilizante estándar. NANO se ha formulado al 100% como fertilizante nanoencapsulado de liberación lenta, teniendo el total de nutrientes solubles en agua para el consumo de las plantas. Este nanoproducto es elaborado con nitrato de amonio y está completamente libre de urea.

Se considera que el uso de este insumo es seguro, eficaz y económico con una reducción del volumen de fertilización total aplicado (4).

En el ámbito agrícola la producción mundial de alimentos ha sido facilitada por las más diversas tecnologías de producción. Uno de los principales factores para el aumento de producción y disminución de costos, es el aprovechamiento máximo y selección especial

de las "mejores" semillas, desarrolladas biotecnológicamente y mediante técnicas genéticas (72).

Por otro lado, respecto del uso de NPs en el área de los fertilizantes químicos o tradicionales, en China se ha reportado la preparación de nanocompuestos que contienen fertilizantes de liberación lenta, lo cual es un avance importante en la nutrición vegetal (39).

Actualmente se considera que los nanodispositivos tendrán la capacidad de detectar una infección por hongos o bacterias, la deficiencia en el uso de nutrientes por las plantas, o cualquier otro problema de fitosanidad, mucho antes de que los síntomas sean evidentes en la escala macro (67). Este tipo de tratamiento con nanocompuestos puede ser dirigido a la zona afectada con una mayor conciencia sobre los riesgos asociados con el uso de plaguicidas sintéticos o tradicionales.

Debido al efecto adverso en los agroecosistemas, se ha originado una necesidad urgente de explorar productos alternativos para el control de plagas y enfermedades (56).

El trabajo de Pérez-de-Luque y Rubiales (2009) pone de manifiesto que la NT está abriendo nuevas aplicaciones potenciales para la agricultura, las cuales ya están siendo exploradas y utilizadas en la medicina y farmacología, pero el interés por su uso en la protección de cultivos apenas está iniciando. El trabajo de esos autores se centra en el desarrollo de nanodispositivos para atacar sitios de las plantas con problemas y para usar nanotransportadores de emisiones químicas controladas.

La NT también puede mejorar las técnicas del manejo de cultivos existentes en el corto y mediano plazo. Las nanocápsulas ayudarán a evitar problemas en los cultivos mediante la utilización de herbicidas sistémicos contra malezas parásitas (83).

La nanoencapsulación puede mejorar la aplicación de herbicidas, proporcionando una mejor penetración a través de cutículas, estomas y tejidos, ya que permite la liberación lenta y constante de las sustancias activas.

Con la nanobiotecnología se plantea la posibilidad de diseñar plantas mediante la manipulación de las semillas (3). Las investigaciones en este campo se basan en el desarrollo de nuevas técnicas que utilizan NPs que permiten introducir ADN ajeno a una célula. Por ejemplo, los investigadores del laboratorio Oak Ridge en Estados Unidos, descubrieron una técnica de escala nanométrica para inyectar ADN de manera simultánea a millones de células. Con esto se ha logrado que millones de nanofibras de carbono con ADN sintético adherido crezcan de un chip de silicio. Posteriormente se lanzan las células vivas contra las fibras que las perforan y les inyectan ADN en el proceso (51).

La conversión directa de la energía solar a electricidad a partir de las proteínas responsables de la fotosíntesis en las hojas de las plantas, es otro ejemplo de una nueva aplicación de las NPs en la agricultura, tal y como lo han demostrado investigadores de la Universidad Vanderbilt en EUA.

Por su parte Scott y Chen (2013) señalan que los cultivos agrícolas no solo pueden incrementar el rendimiento por el uso de la NT, sino que también los nuevos materiales y dispositivos de diseño pueden prevenir la presencia de plagas y enfermedades. De hecho, se prevé un gran futuro a través de la exploración y explotación de los estudios a nanoescala de materiales biológicos de origen agrícola y natural en beneficio de una sociedad sustentable y amigable con el ambiente (15, 48).

EFFECTO DE ALGUNAS NANOPARTÍCULAS EN EL CRECIMIENTO DE LAS PLANTAS

Algunos autores mencionan que el efecto promotor o inhibidor del crecimiento de las NPs en las plantas está relacionado con su concentración, tamaño y las propiedades inherentes del elemento involucrado, así como la función fisiológica y bioquímica que desempeña en la planta, y si actúa como micronutriente tratándose de cobre, zinc, etc. (80).

NPs como las de CuO muestran un efecto positivo sobre la reactividad de fitohormonas, especialmente del ácido indolacético (AIA), así como del ácido salicílico, esto hace que se facilite o incremente la acción fitoestimulante de las NPs (80).

Las NPs de óxido de zinc (NPs ZnO) son usadas en diferentes áreas como la industria de cosméticos, textiles, medicina, electricidad y en la agricultura (8).

En el sector agrícola son estudiadas por su actividad antimicrobial (14, 66) y por su potencial como nanofertilizantes, corrigiendo las deficiencias de zinc en las plantas y promoviendo crecimiento y desarrollo (9, 43, 60). Algunos estudios señalan que concentraciones elevadas (1000 mg L⁻¹) causan fitotoxicidad e inhibición de la germinación (32, 84); mientras que dosis bajas (< 50 mg L⁻¹) han demostrado efectos significativos en el crecimiento y desarrollo (44, 55), reflejándose en mayor biomasa seca y área foliar. Este efecto promotor ha sido atribuido al zinc, por ser este uno de los micronutrientes esenciales demandados para la división celular y por su importancia como componente de varias enzimas en las plantas (47); del mismo modo está implicado en la síntesis de proteínas, carbohidratos, lípidos y ácidos nucleicos (76).

Trabajos efectuados en diversas especies de plantas confirman que las NPs ZnO promueven mayor germinación y crecimiento de plántulas (73).

En el reporte de Panwar *et al.* (2012) se señala más crecimiento y producción de biomasa seca en plántulas de tomate cuando aplicaron 20 mg L⁻¹ al follaje; además, encontraron altas concentraciones de Zn en las hojas, confirmando con ello que ocurrió la penetración en los estomas y su traslocación basipétala vía floema.

Otro estudio realizado por Prasad *et al.* (2012), revela que una concentración de 1000 mg L⁻¹ de NPs ZnO promueven la germinación en semillas de cacahuate, así como la elongación de raíz y tallo. Zhao *et al.* (2014), obtuvieron incrementos significativos en longitud y biomasa seca de la raíz en plantas de pepino cultivadas en maceta, debido a incorporación al suelo de 400 y 800 mg kg⁻¹ de NPs ZnO. Estos investigadores consignan que las altas concentraciones usadas por ellos no afectaron negativamente a las plantas.

En pruebas de germinación de semillas las dosis de 250 a 2000 mg L⁻¹ de NPs ZnO promovieron incrementos significativos de clorofila y proteína en plántulas de trigo (57). Concentraciones bajas (10 y 20 mg L⁻¹) de estas mismas NPs mejoraron la germinación de semillas de cebolla (58).

En plantas de goma guar (*Cyamopsis tetragonoloba* L.) una concentración de 10 mg L⁻¹ de NPs ZnO aplicada foliarmente promovió mayor producción de biomasa, contenido de clorofila, longitud y área radicular, así como síntesis de proteína (57). En plantas de soya la dosis de 1.0 g L⁻¹ de NPs ZnO causó efectos significativos en la germinación y crecimiento (68).

La aplicación foliar en plantas de garbanzo (*Cicer arietinum*) de 1.5 y 10 mg L⁻¹ de NPs ZnO durante 15 días, originaron mayor altura y biomasa seca (47).

Se ha destacado que la aplicación de zinc en forma de nanofertilizante aplicado al follaje en dosis bajas, es más eficiente para promover el crecimiento vegetativo. Se ha sugerido que este efecto se debe a que el zinc es requerido para la producción de biomasa y también porque este microelemento tiene una función muy importante en muchas enzimas involucradas en el proceso fotosintético, así como en la integridad y mantenimiento de las membranas celulares de las plantas (2, 5).

El zinc como nanofertilizante en dosis de 10 mg L⁻¹ ha promovido más crecimiento en plantas de mijo (*Pennisetum americanum*), mayor producción de biomasa seca, longitud de raíz, contenido de clorofila y rendimiento de grano (76).

La aplicación foliar de nanoquelato de zinc como fertilizante promovió un incremento significativo en la altura de plantas de algodón (*Gossypium hirsutum*), así como de biomasa seca y contenido de clorofila (63).

Existen reportes indicando que las NPs ZnO incrementan el nivel de AIA en raíces y en los brotes apicales, incrementando de esta forma la velocidad de crecimiento en las plantas (72).

El efecto de las NPs ZnO en el crecimiento vegetal, podría relacionarse a la actividad que tiene el zinc como precursor en la producción de auxinas reguladoras del crecimiento, las cuales también promueven la elongación y división celular (62).

Además, se reporta que el zinc es uno de los micronutrientes esenciales y un componente muy importante de varias enzimas responsables de muchas reacciones metabólicas (72), igualmente desempeña una importante función en la elaboración de clorofila; germinación de semillas; producción de polen y biomasa (47).

Por otra parte, se ha puntualizado que las nanopartículas de plata (NPs Ag) en concentraciones bajas poseen un efecto positivo en la germinación de semillas y en la promoción del crecimiento en plantas. Sharma *et al.* (2012), reportan que las NPs Ag causan más crecimiento de plántulas de mostaza (*Brassica juncea*) en concentraciones de 25 y 50 mg L⁻¹, reflejándose esto en mayor longitud de raíz, biomasa seca y altura. Sin embargo, altas concentraciones (250-500 mg kg⁻¹ de suelo), inhibieron la germinación y crecimiento en las plantas de haba. Estos incrementos podrían estar relacionados con la producción endógena de fitohormonas como citoquininas y giberelinas, las cuales están implicadas en la división y elongación celular, respectivamente. Además estas NPs pueden mejorar la eficiencia de intercambio de electrones a nivel celular en las plantas, lo que podría reducir la formación de las dañinas especies reactivas de oxígeno (9).

Otras NPs como las de TiO₂ aplicadas al follaje, revelan un incremento en la actividad de varias enzimas y promueven la absorción del nitrato, el cual acelera la transformación del nitrógeno inorgánico a orgánico haciéndolo más asimilable, reflejándose esto en mayor crecimiento vegetal (7).

Estudios realizados por García *et al.* (2011), revelan que las NPs de cerio son extremadamente tóxicas en el conjunto de ensayos realizados, habiendo determinado que en pruebas de germinación, algunas semillas mostraron un efecto perjudicial (fitotoxicidad) en la germinación.

Reportes previos muestran que la fitotoxicidad causada por algunas NPs genera un comportamiento impredecible e irregular sobre el estrés oxidativo, que a su vez depende del tipo, concentración, propiedades y medios de exposición de las NPs (17, 33, 75). Por lo tanto, la función de las NPs en la modulación del sistema de defensa

antioxidante en las plantas no está totalmente elucidado, siendo un tema necesario para ser investigado con mayor profundidad.

Estos resultados pueden servir de base para considerar el uso de NPs magnéticas como un sistema acarreador de ingredientes para remediar enfermedades bacterianas. Por otro lado, Zhu *et al.* (2008), han mencionado que las plantas de calabaza (*Cucurbita maxima*), al aplicarles NPs de magnetita (Fe_3O_4), pueden absorber, traslocar y acumular las NPs en el tejido vegetal. Eso pone de manifiesto que las plantas son un importante componente del medio ambiente y de los hábitats, por lo tanto, se deben considerar e investigar cuando se está evaluando el destino, medio de transporte y caminos que siguen las NPs en las plantas y los ecosistemas.

MODO DE ABSORCIÓN Y TRANSLOCACIÓN DE LAS NANOPARTÍCULAS

Cuando las NPs son aplicadas al follaje se absorben a través de los estomas y su translocación o transporte basipétalo hacia la base del tallo es por el sistema vascular del floema (figura 4). Al ser aplicadas al suelo o en el agua de riego penetran a través de la epidermis de la raíz y la corteza, posteriormente pasan a la endodermis y finalmente entran al tejido conductivo del xilema, para luego ser translocados a larga distancia hasta las ramas y el follaje de las plantas (52, 65).

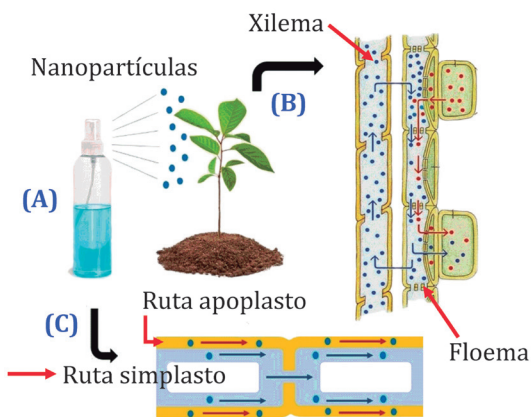


Figura 4. (A) Esquema que ilustra la aplicación de nanopartículas al follaje de las plantas. (B) Cuando penetran a través de los estomas se translocan o transportan vía floema. (C) Cuando se aplican a la zona radicular, su translocación a larga distancia es principalmente por el apoplasto del tejido conductivo del xilema.

Figure 4. (A) Diagram illustrating the application of nanoparticles to plant foliage. (B) When they are absorbed through stomata are translocate or transported via phloem. (C) When applied to the root zone the long distance translocation is primarily by the apoplast conductive xylem tissue.

Se ha señalado que las paredes y membranas celulares actúan como una eficaz barrera a la entrada de cualquier tipo de NPs, y que la efectividad de su entrada y transporte está determinado por el tamaño de los poros de la pared celular, que están en el rango de 5 a 20 nm (13, 16). Por lo tanto, solo NPs de un tamaño menor al de los poros de la pared celular, pudiesen pasar con facilidad y penetrar las membranas en su camino al interior del citoplasma celular.

Los nanomateriales son considerados un factor de estrés en las plantas, ya que existe la posibilidad de que puedan remodelar y modificar la estructura y constitución de las membranas y pared celular en plantas (37).

Diversas investigaciones han demostrado que tanto las NPs de carbono, como las metálicas, son capaces de producir estrés en las plantas, generando un exceso de ERO (especies reactivas del oxígeno) que pueden afectar las proteínas, lípidos, carbohidratos y el ADN. Las NPs alteran la eficiencia fotosintética, la fluorescencia fotoquímica y el rendimiento cuántico en las plantas, debido a las interacciones de estas con los fotosistemas I y II, ya que diversos estudios han demostrado que las clorofilas transfieren la energía a las NPs (46, 64).

A su vez, la perturbación en la actividad fotosintética se traduce en estrés oxidativo en las plantas. El estrés oxidativo en plantas provocado por las NPs se ha investigado a través de técnicas que miden la producción de ERO como H_2O_2 , la activación de los mecanismos de defensa enzimáticos, la peroxidación lipídica y pérdida de electrolitos, entre los más importantes. Sin embargo, aún no se entiende completamente cómo las propiedades químicas de las NPs inducen la producción de ERO y el daño causado a la membrana de las plantas (24).

CONCLUSIONES

La nanotecnología es el conocimiento emergente del siglo XXI en todos los campos de la ciencia. En la agricultura su beneficio incluye el mejoramiento genético de plantas, la mejora de la productividad agrícola utilizando NPs como inductores del crecimiento vegetal, producción de nanoencapsulados para la lenta liberación de fertilizantes, así como para la formulación de nanoplaguicidas y nanoherbicidas.

Con el empleo de la NT también se podrán fabricar nanosensores muy eficaces para la detección temprana de enfermedades. Además se podrá realizar la transferencia de ADN en las plantas para el desarrollo de variedades resistentes a plagas y enfermedades, así como a factores bióticos y abióticos. Sin embargo, la presencia de NPs en cualquiera de sus formas es factible que afecten la estructura de la comunidad microbiana, lo que puede causar alteraciones en la función de los agroecosistemas y comprometer los procesos de producción de alimentos.

La biodisponibilidad del exceso de nanomateriales amenaza en última instancia el ecosistema y la salud humana.

En los últimos años, el campo de la nanotoxicología que estudia los efectos adversos y el riesgo probable asociado con partículas <100 nm, ha surgido de la comprensión reconocida de los efectos tóxicos de las partículas metálicas y otras y sus interacciones con las plantas. Quizá la mayor importancia del uso de la NT en la agricultura incluye las aplicaciones específicas para formular nanofertilizantes y nanopesticidas, que permitan aumentar de manera sustentable la productividad de los cultivos, al reducir la contaminación de los agroecosistemas y coadyuven en la protección contra plagas de insectos y enfermedades microbianas.

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Conclusiones

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